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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Glufosinate-Ammonium (Ignite[R]): Review of toxicity studies on the metabolites and the L-form of glufosinate ammonium

Tox. No. 580I **PC Code**: 128850
Submission No. S509558 **DP Barcode**: D229929

TO: E. Wilson/J. Miller, PM Team 23
Herbicide Branch
Registration Division (7505C)

FROM: Whang Phang, Branch Senior Scientists
Reregistration Branch I/ HED (7509C) 6/10/98

THROUGH: Michael Metzger, Branch Chief
Reregistration Branch I/ HED (7509C) for 6/12/98

Introduction

The registrant, AgrEvo (A Co. of Hoechst and NOR-AM), submitted a series of studies on the metabolites of glufosinate ammonium. These studies are submitted for the purpose of verifying the values of LOEL and NOEL which were presented to the Agency in a pre-registration meeting concerning the selective use of glufosinate ammonium on corn & soybeans. Originally, it was planned to quickly examine the validity and the rationale for establishing the NOEL and the LEL of this series of studies. After a preliminary review of these studies it was decided to have a Data Evaluation Report (DER) prepared for essentially all the studies (except **the acute toxicity studies**) because some of these studies appear to provide useful information on these chemicals. Particularly, the registrant may wish to register the L-form of glufosinate ammonium later. These studies are the following:

MRID	TEST COMPOUND	STUDY TYPE
44067801	Hoe 099730	Acute oral toxicity study in the rats
44067802	Hoe 061517	Acute oral toxicity study in the rats
44076201*	Hoe 099730	Subchronic toxicity study in the rats
44076202*	Hoe 099730	Subchronic toxicity study in the mice
44076203*	Hoe 099730	Subchronic toxicity study in the dogs
44076204	Hoe 099730	Developmental toxicity study in the rats
44076205	Hoe 099730	Developmental toxicity study in the rabbits
44076206*	Hoe 061517	Subchronic toxicity study in the rats
44076207*	Hoe 061517	Subchronic toxicity study in the mice

MRID	TEST COMPOUND	STUDY TYPE
44076208*	~~Hoe~~ 061517	Subchronic toxicity study in the dogs
44076209	Hoe 061517	Developmental toxicity study in the rats
44076210	Hoe 061517	Developmental toxicity study in the rabbits

*: All these subchronic toxicity studies wee reviewed and transmitted to RD on March 7. 1997 (Tox. Doc. No. 012180).

DISCUSSION

As indicated above that every submitted study had been reviewed. The citation of each study and the conclusion of each review are summarized and organized into acute, subchronic, and developmental toxicity studies.

ACUTE ORAL TOXICITY STUDIES

There are two acute oral toxicity studies (MRID No. 44067801 & 44067802) in this data set. The DER's are not prepared for these two studies because they are submitted for verification purposes. However, these two studies have been reviewed, and the conclusion and relevant details for each study are presented below.

1. Schollmeier, U & Leist, KH (1988). Hoe 099730-substance technical (Code: Hoe 099730 0Q ZC83 0001) testing for acute oral toxicity in the male and female Wistar rats. Pharma Research Toxicology and Pathology, Hoechst Aktiengesellschaft; Study No. 88.1547; Report No. A41793. Dec. 15, 1988. **MRID 44067801.** Unpublished.

In an acute oral toxicity study (MRID 44067801), groups of Wistar rats (5/sex/group) received a single dose (2895 mg/kg) of Hoe 099730 (82.6% a.i.) by gavage at a volume of 10 ml/kg b.wt.. The test animals were observed for 14 days. The clinical signs of reduced spontaneous activity, high-legged gait, contracted flanks, squatting position, piloerection, irregular breathing, and increased respiratory rate were seen in both males and females on the day of treatment. These signs were not seen subsequent to the first day of treatment. No deaths occurred at this dose level. Therefore, **the oral LD_{50} was greater than 2895 mg/kg; Toxicity Category III.**

2. Rupprich, N & Weigand, W (1984). Hoe 061517-substance technical (Code: Hoe 061517 0Q ZC99 0002) testing for acute oral toxicity in the male and female Wistar rats. Pharma Research Toxicology and Pathology, Hoechst Aktiengesellschaft; Study No. 84.0265; Report No. A33227. May 23, 1984. **MRID 44067802.** Unpublished

In an acute oral toxicity study (MRID 44067802), groups of Wistar rats received a single dose of Hoe 061517 (99% a.i.) by gavage; the dose levels, volume of administration, and the number of test animals in each group are presented in Table 1. The test animals were observed for 14 days. Immediately after the administration of the test compound, the

following clinical signs were observed in all test animals: squatting, contracted flanks, uncoordinated gait, piloerection, drowsiness, narrowed eye openings, and irregular respiration. Some animal also showed signs of skin pallor, noisy respiration, and widening of the palpebral fissures. Most of these signs were more pronounced at higher dose levels. By day 7 after treatment, essentially all the clinical signs were disappeared from all test animals. The data on death rates are presented in Table 1. Death began to occur at 1600 mg/kg for females (1/5) and 2500 mg/kg for males (2/5). All females in 3150 mg/kg and all males in 5000 mg/kg groups died. **The oral LD_{50} was 2840 mg/kg for males and 1900 mg/kg for females. Toxicity Category III.**

Table 1. Study design and mortality of oral toxicity study on Hoe 061517.

Dose mg/kg b.wt	Volume ml/kg b.wt.	Number of Test Animals		Mortality	
		Male	Female	Male	Female
1250	5.0	5	5	0/5	0/5
1600	6.4	-	5	-	1/5
2000	8.0	5	5	0/5	3/5
2500	10.0	5	-	2/5	-
3150	12.6	5	5	3/5	5/5
5000	20.0	5	-	5/5	-

SUBCHRONIC TOXICITY STUDY

Six subchronic toxicity studies had been reviewed and transmitted to you on March 7, 1997, and to have a closure of this task, the citation of each study and the summary of each review are included. However, the Data Evaluation Records (DER) for these six studies will not be retransmitted.

HOE 099730

1. Tennekes, H., Probst, D., Luetkemeier, H., et al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) Sub-Chronic Oral Toxicity - 13 Week Feeding Study in Rats - Plus Amendment. RCC, Research & Consulting Co., Ltd., Itingen, Switzerland; BRL Biological Research Laboratories, Ltd., Füllinsdorf, Switzerland; EPS (UK) Ltd., Hereford, England; & RCC (UK) Ltd., Hereford, England. Study No. 291093; Report Nos. A48187 & A53387; Study Completion Date: June 28, 1991; Supplement - December 1, 1994; **MRID No.:44076201**. Unpublished.

In a subchronic toxicity study (MRID No.44076201), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 400, 2000 or 10,000 ppm (equivalent daily intakes were 0, 29, 147 or 738 mg/kg/day, respectively in males and 0, 32, 162 or 800 mg/kg/day, respectively in females) to Wistar rats. Groups consisted of 20 rats/sex/dose in the vehicle control and mid- and high-treatment groups and 10 rats/ sex in the low-treatment group. Ten rats/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, food consumption, hematology, clinical chemistry, or urinalysis or cause any gross or microscopic lesions. **The LOEL for glutamine synthetase inhibition is 400 ppm, based on the significant ≈27 or 16% reduction in the activity of this enzyme in male and female livers, respectively, and the ≈20 % reduction in the activity of this enzyme in male kidneys. A NOEL was not established.**

This subchronic toxicity study is classified **Acceptable/Nonguideline** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

2. Tennekes, H., Schmid, H., Probst, D., Luetkemeier, H., et al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) Sub-Chronic Oral Toxicity - 13 Week Feeding Study in Mice - Plus Supplement. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ag, Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; EPS (UK) Ltd., Hereford, England; RCC (UK) Ltd., Hereford, England. Study No. 291025; Report Nos. A48186 & A53386; Study Completion Date: June 10, 1991; Supplement - December 1, 1994; **MRID No.:44076202.** Unpublished.

In a subchronic toxicity study (MRID No.44076202), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 500, 2000 or 8000 ppm (equivalent daily intakes were 0, 83, 324 or 1296 mg/kg/day, respectively in males and 0, 110, 436 or 1743 mg/kg/day, respectively in females) to NMRI mice. Groups consisted of 20 mice/sex/dose. In addition to standard testing, samples of livers, kidneys and brains of all animals were also analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, food consumption, hematology, or clinical chemistry or cause any gross or microscopic lesions. **The NOEL is >8000 ppm (1296 and 1743 mg/kg/day for ♂ & ♀, respectively)** base on the absence of a significant toxicological response at the highest dose level.

The LOEL for glutamine synthetase inhibition is 500 ppm, based on the significant

($p<0.01$) ≥25% reduction in the activity of this enzyme in male an d female kidneys. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable/Nonguideline** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

3. Corney, S.J., Braunhofer, H., Luetkemeier, H., et. al. (1994); Hoe 099730 - Substance Technical (Code: Hoe 099730 00 ZC75 0001) 13-Week Oral Toxicity (Feeding) Study in the Dog - Plus Supplement. RCC, Research & Consulting Co., Ltd., Itingen, SW; RCC Umweltchemie, Ag., Itingen, SW; BRL Biological Research Laboratories, Ltd., Füllinsdorf, SW; RCC (UK) Ltd., Hereford, England. Study No. 291104; Report Nos. A49064 & A53388; Study Completion Date: October 27,1992; Supplement - December 1, 1994; **MRID No.:44076203.** Unpublished.

In a subchronic toxicity study (MRID No.44076203), Hoe 099730 (44.4% solution in water, adjusted to 100% purity) was administered in the diet at dose levels of 500, 2000 or 8000 ppm (equivalent daily intakes were 0, 19, 72 or 289 mg/kg/day, respectively in males and 0, 21, 79 or 300 mg/kg/day, respectively in females) to beagle dogs. Groups consisted of six dogs/sex/dose in the vehicle control and mid- and high-treatment groups and four dogs/sex in the low-treatment group. Two dogs/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were also analyzed for glutamine synthetase activity.

Treatment with Hoe 99730 had no adverse effects on survival, body weight, hematology, or clinical chemistry or cause any gross or microscopic lesions. **The NOEL is >8000 ppm (289 & 300 mg/kg/day for ♂ & ♀, respectively)** base on the absence of a significant toxicological response at the highest dose level.

The LOEL for glutamine synthetase inhibition is 500 ppm, based on the significant ($p<0.01$) ≈31% reduction in the activity of this enzyme in male livers. A NOEL was not established.

This subchronic toxicity study is classified **Acceptable/Nonguideline** as it is not a required study. It is acceptable for the purposes for which it was intended as a special study.

4. Ebert, E. and Mayer, D. (1988); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) Subchronic Oral Toxicity (13-Week Feeding Study) in the Wistar Rat. Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 831 & 87.0721: Report No. A40450; Study Completion Date: March 30, 1988; **MRID No.:44076206.** Unpublished.

In a subchronic toxicity study (MRID No.44076206), Hoe 061517 (99.6%) was administered in the diet for 13 weeks to male and female Wistar rats at dose levels of 0, 400, 1600 or 6400 ppm (0, 30, 102 or 420 mg/kg/day, respectively in males and 0, 32, 113, or 439 mg/kg, respectively in females). Groups consisted of 20 rats/sex/dose in the vehicle control and mid- and high-treatment groups and 10 rats/sex in the low-treatment group. Ten rats/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, neurological examinations were conducted; however, glutamine synthetase activity was not measured.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology, clinical chemistry, urinalysis, or cause any neurological changes. However, marginal increases in the absolute and relative high-dose male liver weights (main and recovery groups), which appeared to correlate with the increased incidence of small Kupffer cell proliferates (≈60% of the animals--both main and recovery high-dose groups versus 20% of controls) and increased reticulocyte counts (25% or 14%), were seen at 6400 ppm. Whether these findings are indicative of a toxicologically significant adverse effect is not clear. **Based on the above findings and in disagreement with the study authors, the LOEL was set at 6400 ppm. The NOEL was established at 1600 ppm.**

This subchronic toxicity study is classified **acceptable (Nonguideline)** as it is not a required study. It is, however, acceptable for the purposes for which it was intended as a special study.

5. Ebert, E. and Leist, K.H. (1989); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0005) Subchronic Oral Toxicity (13 Week Feeding Study) in the NMRI Mouse. Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 88.0693: Report No. A41762; Study Completion Date: June 16, 1989; **MRID No.:44076207.** Unpublished.

In a subchronic toxicity study (MRID No.44076207), Hoe 061517 (99.8%) was administered in the diet for 13 weeks to male and female NMRI mice at dose levels of 0, 320, 1600, 3200 or 8000 ppm (0, 46, 209, 496 or 1121 mg/kg/day in the males and 0, 47, 220, 561 or 1340 mg/kg/day in the females, respectively. All groups consisted of 10 mice/sex/dose. Glutamine synthetase activity was not measured.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology or urinalysis. However, a marginal but significant increase in the relative high-dose female kidney weight and dose-related significant decreases in serum uric acid levels were observed in the mid- and high-dose males (16 and 40% of control, respectively). In the females, non-dose-related ≈33 or 25% decreases in uric acid levels were seen at 3200 or 8000 ppm, respectively. There was, however, no evidence of gross or microscopic lesions

associated with exposure to Hoe 061517. Whether the marginal kidney weight changes or decreased uric acid levels are indicative of a toxicologically significant adverse effect is not clear. **In disagreement with the study authors, however, the LOEL was set at 8000 ppm based on the decreased serum uric acid levels. The NOEL was established at 3200 ppm.**

This subchronic toxicity study is classified **acceptable/Nonguideline** as it is not a required study. It is, however, acceptable for the purposes for which it was intended as a special study.

6. Brunk, R. (1988); Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) Testing for Toxicity by Repeated Oral Administration to Beagle Dogs (3-Month Feeding Study). Pharma Research Toxicology and Pathology Hoechst Aktiengesellschaft, Frankfurt am Main, Germany. Study No. 87.0722: Report No. A39880; Study Completion Date: June 21, 1988; **MRID No.:44076208.** Unpublished.

In a subchronic toxicity study (MRID No.44076208), Hoe 061517 (99.6%) was administered in the diet for ≈15 weeks to male and female beagle dogs at dose levels of 100, 400 or 1600 mg/kg (equivalent daily intakes could not be determined because of the inadequacies in the analytical data). Groups consisted of six dogs/sex/dose in the vehicle control and mid- and high-treatment groups and four dogs/sex in the low-treatment group. Two dogs/sex/dose from the vehicle control and mid- and high-treatment groups were retained following treatment for a 4-week recovery period. In addition to standard testing, samples of livers, kidneys and brains of all animals were analyzed for glutamine synthetase activity. Hearing tests, neurological examinations hepatic function tests (i.e., bromsulphthalein retention) and renal function test (i.e., phenolsulfonphthalein elimination) were also conducted.

Treatment with Hoe 061517 had no adverse effects on survival, body weight, hematology, clinical chemistry, urinalysis, liver or renal function, glutamine synthetase activity, or cause hearing disfunction or neurological changes. Similarly, there was no evidence of gross or microscopic lesions associated with exposure to Hoe 061517. **However, neither a LOEL nor a NOEL could be established because of numerous study deficiencies (see Section III, Discussion for details).**

This subchronic toxicity study is classified **Unacceptable (Nonguideline)** as it is not a required study. It is, however, unacceptable for the purposes for which it was intended as a special study.

DEVELOPMENTAL TOXICITY STUDIES

1. Horstmann, G. & Baeder, C. (1993) HOE 099730 -substance Technical (Code: Hoe 099730 00 AC75 00001): Testing for Embryotoxicity in the Wistar Rat After Oral Administration (Limit Test) Plus Supplement. Pharma Development Central Toxicology, Frankfurt, Germany. Study Nos. RR0628 & 90.1227; Report Numbers A48852 & A51525, September 15, 1992 Supplement on October 28, 1993. **MRID 44076204.** Unpublished.

In a developmental toxicity study (limit test) (MRID 44076204) Hoe 099730 00 ZC75 0001 (74.7% a.i.) a glufosinate ammonium metabolite, was administered once daily via oral gavage to 20-21 female Wister rats at dose levels of 0 or 1000 mg/kg/day from days 7 through 16 of gestation.

No maternal toxicity was observed at the 1000 mg/kg/day dose. There were no treatment-related effects in mortality, body weight, feed consumption, gross pathology, or cesarean section parameters. **The maternal LOEL was not observed. The maternal NOEL is >1000 mg/kg/day.**

There was no evidence of treatment-related developmental toxicity in the treatment group. There were no treatment-related effects found upon cesarean section examinations. Numbers of corpora lutea, implantations, live fetuses, resorptions, and fetal weights were similar among treated and untreated animals. There were no treatment-related effects found upon external, visceral, and skeletal examination of the fetuses. **The developmental LOEL was not observed. The developmental NOEL is >1000 mg/kg/day.**

Dosing was considered adequate because the dose of 1000 mg/kg/day represents a "limit dose". The developmental toxicity study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 (a) in rats.

2. Baeder, C. and Hofmann, T. (1995) Hoe 099730-Substance-Technical (Code: Hoe 099730 ZC92 0001): Testing for Embryotoxicity after Oral Administration in Himalayan Rabbits Plus Supplement, Pharma Development Corporate Technology, Germany. Study No. 93.0112 & RK0669. Laboratory report numbers A52948 & A54431, August 24, 1994 - Supplement on May 26, 1995. **MRID 44076205.** Unpublished

In a developmental toxicity study (MRID 44076205) HOE 099730 (92.4% a.i.) was administered to 15 Himalayan rabbits/dose in distilled water by gavage at dose levels of 0, 64, 160, or 400 mg/kg/day from days 6 through 18 of gestation.

Minimal maternal toxicity was demonstrated by reduced feed consumption (↓22-24%; $p<0.05$) in the 160 mg/kg/day group and in the 400 mg/kg/day group (↓30-40%; $p<0.05$) during treatment days 6-13 and 13-19. There were no treatment-related effects in mortality, clinical signs, body weight, or cesarean section parameters. **The maternal LOEL was 160 mg/kg/day based on reduced feed consumption. The maternal NOEL was 64 mg/kg/day.**

A uni- or bilateral extra rib at the 13th thoracic vertebra was observed in the 160 mg/kg/day group; **based on this finding the developmental LOEL was 160 mg/kg. The developmental NOEL was 64 mg/kg/day.**

This study was submitted for verification of the NOEL and LEL provided by the registrant to show that toxicity of this metabolite is less than that of the parent compound. Under the circumstance, this study is considered as **acceptable/non-guideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in rabbits.

3. Albrecht, M. and Baeder, C., (1994) Testing of Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC 99 0003) for Embryotoxicity in the Wistar Rat After Oral Administration. Pharma Research Toxicology and Pathology, Germany. Study Numbers: RR0545 & 87.0726; Report Number A52161; February 3, 1994. **MRID 44076209**. Unpublished.

In a developmental toxicity study (MRID 44076209) Hoe 061517 (a metabolite of glufosinate ammonium; 99.6% a.i.) in distilled water was administered by gavage to 20 presumed pregnant Wistar rats/dose at dose levels of 0, 100, 300, or 900 mg/kg/day from days 6 through 17 of gestation

Maternal toxicity was demonstrated at 900 mg/kg/day, maternal toxicity was demonstrated by one death, treatment-related clinical findings (persistent piloerection and/or increased urinary output), increased absolute kidney weights. **The maternal LOEL is 900 mg/kg/day; NOEL, 300 mg/kg/day.**

At 900 mg/kg/day, increases in the incidence of total litter loss and in the fetal and litter incidence of wavy and/or thickened ribs were found. There were additional treatment-related effects noted in developmental parameters. **Therefore, the developmental LOEL for developmental toxicity is 900 mg/kg/day; NOEL, 300 mg/kg/day.**

The developmental toxicity study in the rat is classified as **acceptable/non-guideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat. It should be noted that this study was submitted for verification of the NOEL and LOEL provided by the registrant to show that the toxicity of various metabolites is less than that of the parent compound.

4. Albrecht, M. and Baeder, C. (1994) Testing of Hoe 061517 -Substance Technical (Code: Hoe 061517 0Q ZC99 0003) For Embryotoxicity in the Himalayan Rabbit After Oral Administration. Hoechst Aktiengesellschaft, 6230 Frankfurt am Main 80, Germany. Laboratory Study Numbers, RK0546 & 87.0727; Report Number A52160, February 2, 1994. MRID 44076210. Unpublished.

In a developmental toxicity study (MRID 44076210) Hoe 061517 (a metabolite of glufosinate ammonium) (99.6% a.i.) in distilled water was administered to 15 Hoe:HIMK (SPFWiga) Himalayan rabbits/dose/group by gavage at dose levels of 0, 50, 100, or 200 mg/kg/day from days 6 through 18 of gestation.

Maternal toxicity was demonstrated at 100 mg/kg/day, as a dose-related increase in abortions (7% vs. 0% in controls) and mortality (7% vs. 0% in controls), clinical signs (disequilibrium), and reductions in food and water consumption, body weight gain, and fecal output. At 200 mg/kg/day, maternal toxicity was demonstrated by treatment-related clinical signs of toxicity (disequilibrium, and/or straddled fore-limbs), increases in abortions (27% vs. 0% in controls) and mortality (33% vs. 0% in controls), reductions in body weight gain, food and water consumption, and fecal output. In addition, treatment-related gross pathology was noted in the kidneys of the high-dose animals and was characterized as uneven, rough surface of one high-dose dam, and light-brown coloring of the renal cortex of three of the four aborting high-dose dams. Corroborative treatment-related increases in the mean kidney weights was also noted at 200 mg/kg/day.

At 50 mg/kg level, no treatment-related deaths or effects were reported. **The maternal LOEL is 100 mg/kg/day, based on increased abortions and mortality and reductions in food and water consumption, body weight gain, and fecal output. The maternal NOEL is 50 mg/kg/day.**

There were no treatment-related effects noted in developmental parameters at any dose level. **A developmental LOEL was not observed (>200 mg/kg/day). The developmental NOEL is 200 mg/kg/day.**

This developmental toxicity study in the rabbit is classified as **unacceptable** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in the rabbit. In order to upgrade the study, the sponsor must submit data confirming the nominal concentrations of the administered doses and the stability of the test substance in distilled water.

CONCLUSION

The values of the LD_{50} of the acute toxicity studies and NOEL of subchronic and developmental toxicity studies on metabolites of glufosinate ammmonium are summarized and compare to those of the parent compound where possible in Table 2. Based on the results presented in this table, the two metabolites (Hoe 99730 and Hoe 061517), in general, are less toxic than the parent compound.

Table 2. Comparison of the values of LD_{50} and of NOEL from various studies.

Study/parameter	mg/kg/day		
	Hoe 039866	Hoe 099730	Hoe 061517
Rat oral LD_{50} (male/female)	2000/1620	>2895	2840/1900
Mouse oral LD_{50} (male/female)	431/416		
Subchronic toxicity			
90-day rat- NOEL (male/female)	4.1	738/800	102/113
90-day mouse- NOEL (male/female)	16.6	1296/1743	496/561
90-day dog--NOEL (male/female)	0.53	289/300	Can't be established
Developmental Toxicity			
Rats-NOEL (maternal/developmental)	10/50	100/>1000	300/300
Rabbits-NOEL (maternal/developmental	6.3/20	64/64	50/200

A comparison of the toxicity, based on the NOEL values, between the DL-glufosinate ammonium and the L-glufosinate ammonium is shown in Table 3. Based on the data in this submission, the purified L-glufosinate ammonium is more toxic than the DL mixture. This finding is consistent because the L-isomer is the active form of this chemical.

Table 3: Comparative toxicity of DL- and L-glufosinate ammonium[a]

Study Type	mg/kg+	
	DL-GFA*	L-GFA*
Rat oral LD_{50} (male/female)	2000/1620	709/669
Mouse oral LD_{50} (male/female)	431/417	137/129
Rat intraperitoneal LD_{50} (male/female)	96/83	95/20
Rat inhalation LC_{50} (male/female) (mg/L)	1.26/2.60	0.139/0.314
Developmental toxicity-rabbit (NOEL: maternal/developmental)	10/50	1.25/1.25

a: The data for DL-glufosinate ammonium are excerpted from the HED One-liner and the submission.

+: For the rat inhalation study the unit is mg/L.

*: DL-GFA = DL-glufosinate ammonium (Hoe 039866); L-GFA = L-glufosinate ammonium (Hoe 058192).

DATA EVALUATION RECORD 012647

GILUFOSINATE - AMMONIUM (HOE 099730)

Study Type: 83-3(a); Testing for Embryotoxicity in the Wistar Rat After Oral Administration (Limit Test) Plus Supplement

Work Assignment No. 2-37D (MRID 44076204)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 12/28/96

Secondary Reviewer:
Sandra Daussin
Signature:
Date: 12/31/96

Program Manager:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 1/8/97

Quality Assurance:
Michael Norvell, Ph.D.
Signature:
Date: 2/3/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Whang Phang, Ph.D. 3/12/97
Review Section III, Toxicology Branch II (7509C)

EPA Secondary Reviewer: James Rowe, Ph.D. James N. Rowe 3/12/97
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

012647

STUDY TYPE: Prenatal Developmental Study-Rat
OPPTS Number: 870.3700 OPP Guideline Number: §83-3(a)

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580I

TEST MATERIAL (PURITY): Hoe 099730 00 ZC75 0001 (74.7 a.i.%)

SYNONYMS: Glufosinate ammonium metabolite (N-acetyl-L-glufosinate ammonium)

CITATION: Horstmann, G. & Baeder, C. (1993) HOE 099730 - substance Technical (Code: Hoe 099730 00 AC75 00001): Testing for Embryotoxicity in the Wistar Rat After Oral Administration (Limit Test) Plus Supplement. Pharma Development Central Toxicology, Frankfurt, Germany. Study Nos. RR0628 & 90.1227; Report Numbers A48852 & A51525, September 15, 1992 Supplement on October 28, 1993. MRID 44076204. Unpublished.

SPONSOR: AgrEvo USA Company, 2711 Centerville Road, Wilmington, DE

EXECUTIVE SUMMARY:

In a developmental toxicity study (limit test) (MRID 44076204) Hoe 099730 00 ZC75 0001 (74.7% a.i.) a glufosinate ammonium metabolite, was administered once daily via oral gavage to 20-21 female Wister rats at dose levels of 0 or 1000 mg/kg/day from days 7 through 16 of gestation.

No maternal toxicity was observed at the 1000 mg/kg/day dose. There were no treatment-related effects in mortality, body weight, feed consumption, gross pathology, or cesarean section parameters. **The maternal LOEL was not observed. The maternal NOEL is >1000 mg/kg/day.**

There was no evidence of treatment-related developmental toxicity in the treatment group. There were no treatment-related effects found upon cesarean section examinations. Numbers of corpora lutea, implantations, live fetuses, resorptions, and fetal weights were similar among treated and untreated animals. There were no treatment-related effects found upon external, visceral, and skeletal examination of the fetuses. **The developmental LOEL**

was not observed. The developmental NOEL is >1000 mg/kg/day.

Dosing was considered adequate because the dose of 1000 mg/kg/day represents a "limit dose".

The developmental toxicity study in the rat is classified **acceptable/guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 (a) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Hoe 099730 00 ZC75 0001
   Description: Technical, brown aqueous solution
   Lot/Batch #: Op. No. 1+2/90
   Purity: 74.7% a.i.
   CAS #: 77182-82-2

2. Vehicle:
   Description: Distilled water

3. Test animals: Species: rat
   Strain: Hoe:WISKf(SPF71)
   Age at mating: 65-70 days
   Weight at mating: mean of 193 ± 8 g
   Source: Hoechst breeding colony
   Housing: Individual plastic cages on wood-shavings
   Diet: Altromin 1310 diet (Altromin GmbH in D-4937 Lage/Lippe), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 20.5-24 C
   Humidity: 49-53%
   Air changes: 16-20/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 4/2/91 end: 5/6/91

2. Mating: Females in estrus were paired overnight on a 1F:1M basis with sexually mature males. Day 1 of gravidity was designated on the day in which sperm was observed in a vaginal smear. The presence of implantation sites confirmed pregnancy status.

3. Animal Assignment: Animals were randomly assigned to control (5 ml starch mucilage/kg/day) and one dose group as indicated in Table 1.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	21
High (HDT)	1000	20

4. Dose selection rationale: In a range-finding study that was briefly summarized in the present submission, Hoe 099730 was administered orally to groups of 3 female rats each at 111.1 or 1000 mg/kg/day (vehicle not indicated). The test solutions were administered on days 7 through 16 of gestation and on day 21 of gestation the females were sacrificed and the fetuses were delivered by caesarean section. The study authors stated that the test compound was tolerated by the dams and conceptuses without complications; no data were submitted to verify this conclusion.

   Based upon the results of this range-finding study, Hoe 099730 was tested at a dose level of 1000 mg/kg/day in this limit test.

5. Dosage preparation and analysis: Test formulations were prepared daily by mixing appropriate amounts of the test substance with distilled water. The animals were dosed within four hours of test substance preparation. Prior to the start of the study, homogeneity and stability determinations were made for samples taken from each of three levels (top, middle, and bottom) of formulations prepared in water at 250 g/l and 500 g/l concentrations and stored for 4 hours. A concentration analysis of three samples from a formulation prepared at 200 g/l in water was also performed.

   Results - Homogeneity and Stability Analyses: 92.2-101% of nominal.

   Concentration Analysis: 101-103% of nominal.

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered orally once daily by gavage, on gestation days 7 through 16, in a volume of 5 ml/kg of body weight. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for clinical signs of toxicity daily. Body weights were recorded on days 0, 7, 14, 17, and on day 21 of gestation. Body weight gains were determined weekly and one day after the final dosing. Feed consumption data were recorded for days 1-7, 7-14, 14-17, and 17-21 of gestation. Dams were sacrificed on day 21 of gestation and delivered by caesarean section. Examination of the dams consisted of macroscopic examination of organs and weighing of heart,

liver, kidneys, and spleen. The reproductive tract was removed and the following were recorded:
- number of implantation sites
- number of live and dead fetuses
- number and diameter of resorptions (early and late)
- number of corpora lutea
- placental weights

2. Fetal Evaluations - Each fetus was examined for external abnormalities and the sex and crown to rump length was determined. Approximately one-half of the fetuses were fixed in alcohol, dissected, eviscerated, and then cleared (aqueous KOH). The skeletons were stained with Alizarin Red S in preparation for skeletal evaluation. The remaining fetuses were fixed in Bouin's solution, cross-sectioned, and examined for visceral examination according to the method of Wilson (1965). Fetal malformations were classified as major or minor defects, variation, or growth retardation.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: Pre-implantation and post-implantation loss indices were calculated from cesarean section records of animals in the study. The calculations used to determine these indices were not provided.

3. Historical control data: Selected historical control data were provided to allow for comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: All dams survived to termination of gestation on day 21. No treatment related clinical observations were observed.

2. Body Weight - Mean body weight gain of the dosed dams was comparable to that of the controls throughout the study (for example, days 0-21: Controls, 141±15 gm; 1000 mg/kg, 133±17 gm).

3. Feed Consumption - There were slight, but statistically significant decreases in feed consumption by the dosed dams (↓5.1-8.7%; $p<0.05$) on study days 7-14 and 14-17. At the 17-21 day interval, feed consumption by the dosed females was similar to the controls. Because these difference in feed consumption were small and not persistent, and body

weight gain in the dosed females was not affected, this finding was considered to not be of toxicological concern.

4. Gross Pathology - No treatment-related gross pathologic findings were noted in any of the dams. Heart, liver, kidney, and spleen weights of the treated dams were similar to the controls.

5. Cesarean Section Data - Cesarean section observations are found in Table 2. The numbers of corpora lutea, implantations, placental weights, and crown to rump lengths were similar between control and treated groups. Pre- and post-implantation losses in the treated dams were 1.7x and 1.3x, respectively, greater than the controls. However, the number of implantations and live fetuses in the treated and the control dams were similar. In addition, the body weights of the fetuses from the treated group were slightly higher (3%; $p<0.05$) than the concurrent controls but within the range of the historical controls.

Table 2. Cesarean Section Observations.[a]

Observation	Dose (mg/kg/day)	
	0	1000
# Animals Assigned (Mated)	21	20
# Animals Pregnant Pregnancy Rate (%)	20 (95)	20 (100)
# Nonpregnant	1	0
Maternal Wastage # Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	0 0 0 0 0 0	0 0 0 0 0 0
Total # Corpora Lutea Corpora Lutea/Dam	294 14.7±1.3	287 14.4±1.8
Total # Implantation Implantation/Dam	280 14.0±1.1	264 13.2±1.5
Total # Litters	20	20
Total # Live Fetuses Live Fetuses/Dam	272 13.6±1.2	254 12.7±1.8
Total # Dead Fetuses Dead Fetuses/Dam	0 0	0 0
Total # Resorptions Early Late Resorptions/Dam Early Late Litters with Total Resorptions	8 8 0 0.4±0.82 0.4±0.82 0 0	10 10 0 0.5±0.95 0.5±0.95 0 0
Mean Fetal Weight (g)	3.4±0.2	3.5±0.3 *
Sex Ratio (% Male)	52.94	50.79
Crown/Rump Length (mm)	35.3±1.7	35.4±1.7
Preimplantation Loss (%)	4.53	7.71
Postimplantation Loss (%)	2.81	3.79
Placental Weight (g)	0.47±0.08	0.43±0.07

a: Data extracted from the study report pages 80 and 81.
*: $p<0.05$

B. DEVELOPMENTAL TOXICITY - Fetal examinations included external and internal observations of soft tissue at necropsy and post fixed and stained skeletal examinations. The study report classified fetal findings as major or minor defects, variations, or growth retardations and provided a summary incidence of the number of fetuses and litters and mean percents affected in each evaluation category. Table 3 notes the most common findings. There were no treatment-related effects reported at the limit dose.

1. External and Visceral Examination - The fetal and litter incidence of blood in the pericardium and abdominal cavity was higher in the dosed group (fetal: 3.8-4.1%; litter: 20-25%) compared to concurrent controls (fetal: 0.7-1.5%; litter:5-10%). The historical control fetal incidence for blood in the pericardium was 0.0-1.5%. These minor differences were not statistically significant and were not judged to be of toxicological concern. All other fetal findings were also observed at incident rates that were statistically comparable to the concurrent controls.

2. Skeletal Examination - All fetal findings were observed at incident rates that were statistically comparable to the concurrent controls.

Table 3. Summary of [a] noteworthy fetal observations at necropsy.

Observations	Dose (mg/kg/day)		Historical controls [b]
	0	1000	
#Fetuses (litters) examined	132-140 (20)	122-132 (20)	--
EXTERNAL/VISCERAL			
#Normal fetuses-(litters)	134 (20)	122 (20)	--
Minor defects [c]:			
Eye-Half-open eyelid-left	0 (0)	0.8 (5.0)	--
Heart-Blood in pericardium	0.7 (5.0)	3.8 (20.0)	0.0-1.5 (--)
Kidney-distended pelvis, uni- or bilateral	3.6 (25.0)	3.8 (15.0)	-- (--)
ORGAN CROSS-SECTIONING			
#Normal fetuses-(litters)	125 (20)	111 (20)	--
Minor defects [c]:			
Abdominal cavity-blood in cavity	1.5 (10.0)	4.1 (25.0)	--
Kidney/Ureter-distended pelvis, uni- or bilateral; and distended ureter, left or bilateral	2.3 (10.0)	3.3 (20.0)	--

Observations	Dose (mg/kg/day)		Historical controls [b]
	0	1000	
SKELETON			
#Normal fetuses (litters)	30 (13)	58 (18)	--
Minor defects [c]:			
Sternebra- Displaced, dysplasia	2.9 (20.0)	4.5 (25.0)	--
Retarded growth [c]:			
Skull- slight ossification of skull bones	26.4 (65.0)	15.9 (65.0)	--
Caudal Vertebral Centra- <2 centers ossified	17.9 (50.0)	16.7 (55.0)	--
Sternebra- unossified or partially ossified	13.6 (55.0)	13.6 (65.0)	--
Forepaw- Non-ossified metacarpal	37.1 (90.0)	25.8 (70.0)	--
Variation c:			
Extra rib- at 7th cervical vertebra, short, unilateral	0.7 (5.0)	0.8 (5.0)	--
at 1st lumbar vertebra, uni- or bilateral	33.6 (80.0)	19.7 (65.0)	--

a Data extracted from the study report pages 85-89;
-- = not reported.
b The historical control values are for the range of % fetal incidence only.
c Fetal (litter) incidence as %.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS The study report concluded that oral administration of Hoe 099730 at 1000 mg/kg/day to pregnant rats during organogenesis resulted in no maternal or developmental adverse effects.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: The reviewers agree with the study report that following oral administration of the test substance, Hoe 099730 00 ZC75 0001, a glufosinate ammonium metabolite (74.7% a.i.), to pregnant rats on days 7-16 of gestation produced no maternal adverse effects. There were no treatment-related effects on mortality or cesarean

section parameters. There were slight, but statistically significant decreases in feed consumption by the dosed dams (↓5.1-8.7%; $p<0.05$) during the 7-14 and 14-17 day intervals. Feed consumption by the dosed females was similar to the controls at the 17-21 day interval. Because these differences in feed consumption were small and not persistent, and body weight gain by the dosed females was not affected, this finding was considered to be not of toxicological concern.

Maternal NOEL >1000 mg/kg/day
Maternal LOEL = Not observed

2. DEVELOPMENTAL TOXICITY: No evidence of treatment-related effects on fetal development were observed following oral administration of the test substance at any dose level.

   a. Deaths/Resorptions: The numbers of resorption/dam and viable fetuses/dam for the treatment group were not significantly different from the concurrent or historical controls.

   b. Altered Growth and Growth Retardations: The body weights of the fetuses from the treated group were slightly higher (3%; $p<0.05$) than the concurrent controls but within the range of the historical controls. All observed growth retardations, such as decreased ossification of cranial bones, sternebrae, caudal vertebrae, and os metacarpal 5, were found at incidences within the range of the concurrent controls.

   c. Developmental Defects and Variations : All observed defects and variations were found at incidences within the range of the historical and concurrent controls with the exception of blood in the pericardium and abdominal cavity. The fetal and litter incidence of blood in the pericardium and abdominal cavity was higher in the dosed group (fetal: 3.8-4.1%; litter: 20-25%) compared to concurrent controls (fetal: 0.7-1.5%; litter:5-10%). The historical control fetal incidence for blood in the pericardium was 0.0-1.5%. This minor defect was nonsignificant and not treatment-related.

   d. Malformations: All observed malformations were found at percent incidences within the range of the historical and concurrent controls.

   Developmental Toxicity NOEL = 1000 mg/kg/day
   Developmental Toxicity LOEL = Not observed

C. STUDY DEFICIENCIES Gravid uterine weights were not presented; this minor deficiency did not affect the adequacy of the study to determine developmental and maternal toxicity.

The developmental toxicity study (limit test) in rats is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in rats.

DATA EVALUATION RECORD

012647

GLUFOSINATE AMMONIUM (HOE 099730)

Study Type: 83-3b; Testing for Embryotoxicity after Oral Administration in Himalayan Rabbits Plus Supplement

Work Assignment No. 2-37C (MRID 44076205)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 12/13/96

Secondary Reviewer:
Sandra Daussin

Signature: Sandra Daussin
Date: 12/15/96

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 1/8/97

Quality Assurance:
Michael Norvell, Ph.D.

Signature: Michael Norvell
Date: 01/07/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Whang Phang, Ph.D.
Reregistration Branch I/HED (7509C) Whang Phang 2/12/98

EPA Secondary Reviewer: Susan Makris, M.S. Susan J Makris 6/2/98
Toxicology Branch I/HED(7509C)

012647

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rabbit
(§83-3(b);OPPTS 870.3700)

DP BARCODE:	D229929	SUBMISSION CODE:	S509558
P.C. CODE:	128850	TOX. CHEM. NO.:	580I
MRID:	44076205		

TEST MATERIAL (PURITY): HOE 099730 00 ZC92 0001, technical (92.4% a.i.) (a metabolite of glufosinate ammonium)

SYNONYMS: HOE 099730; N-acetyl-glufosinate ammonium.

CITATION: Baeder, C. and Hofmann, T. (1995) Hoe 099730-Substance-Technical (Code: Hoe 099730 ZC92 0001): Testing for Embryotoxicity after Oral Administration in Himalayan Rabbits Plus Supplement, Pharma Development Corporate Technology, Germany. Study No. 93.0112 & RK0669. Laboratory report numbers A52948 & A54431, August 24, 1994 - Supplement on May 26, 1995. MRID 44076205. Unpublished

SPONSOR: AgrEvo USA Company, 2711 Centerville Road, Wilmington, DE

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44076205) HOE 099730 (92.4% a.i.) was administered to 15 Himalayan rabbits/dose in distilled water by gavage at dose levels of 0, 64, 160, or 400 mg/kg/day from days 6 through 18 of gestation.

Minimal maternal toxicity was demonstrated by reduced feed consumption (↓22-24%; $p<0.05$) in the 160 mg/kg/day group and in the 400 mg/kg/day group (↓30-40%; $p<0.05$) during treatment days 6-13 and 13-19. There were no treatment-related effects in mortality, clinical signs, body weight, or cesarean section parameters. **The maternal LOEL was 160 mg/kg/day based on reduced feed consumption. The maternal NOEL was 64 mg/kg/day.**

A uni- or bilateral extra rib at the 13th thoracic vertebra was observed in the 160 mg/kg/day group; **based on this finding the developmental LOEL was 160 mg/kg. The developmental NOEL was 64 mg/kg/day.**

Usually, data are required to confirm the nominal concentrations of the administered doses. Without these data, the study would have been classified as unacceptable. However, the test substance is a metabolite of glufosinate ammonium which has an adequate developmental toxicity data base. In addition, this study was submitted for verification of the NOEL and LEL provided by the registrant to show that toxicity of various metabolites is less than that of the parent compound. Under the circumstance, this study is considered as **acceptable/nonguideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: HOE 099730 00 ZC92 0001
   Description: technical, brown liquid
   Lot/Batch #: Lot 2+3 (Fass 1-4)
   Purity: 92.4% a.i.
   CAS #: Not provided
2. Vehicle: Distilled water
3. Test animals: Species: Rabbit
   Strain: Himalayan HIMK(SPFWiga)
   Age at mating: 8-10 months
   Weight at mating: Mean weights at beginning of gestation were 2536-2613 g
   Source: Breeder was Karl Thomae GmbH
   Housing: individually, except when mated (two animals/cage), in steel Type HD 3 cages
   Diet: Pelleted Altromin 2123 rabbit diet (Altromin GmbH, Lage/Lippe, FRG), ad libitum and hay (40-50 g/day)
   Water: tap water, ad libitum
   Environmental conditions:
   Temperature: approximately 22-23 C
   Humidity: 44-74%
   Air changes: 16-20/hr
   Photoperiod: 10 hrs dark/14 hrs light
   Acclimation period (P): at least 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 7/14/93, end: 9/2/93

2. Mating: Each female was mated with a mature male rabbit in the ratio of 1 male:1 female. Animals with sperm in the vaginal smear were mated again after 6 hours to assure that a successful mating occurred. Day 0 of gestation was designated as the day of mating.

3. Animal Assignment: Animals were assigned to treatment groups as indicated in Table 1 using a computer-generated algorithm.

Table 1. Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	15
Low (LDT)	64	15
Mid (MDT)	160	15
High (HDT)	400	15

4. Dose selection rationale: In a range-finding study summarized in the current submission, HOE 099730 (% a.i. not indicated) was administered daily via oral gavage to 2 pregnant female rabbits/dose at dosages of 8.88, 320, 500, 750, or 1,000 mg/kg/day on gestational days 6 through 18. Animals were sacrificed on day 29 of gestation and caesarean sections were performed.

There were no treatment-related findings in either the does or the fetuses at the 8.88 mg/kg/day dose. Dose related maternal toxicity consisting of reduced feed and water consumption and body weight gain, as well as reduced defecation and pultaceous feces, were observed in the 320, 500, and 750 mg/kg/day females. In the females dosed at 1,000 mg/kg/day, there were marked decreases in body weight gain and feed and water consumption as well as vaginal bleeding. One high-dose female was killed on day 22. The second high-dose female delivered one live fetus on day 26 and its uterus contained three live fetuses, four markedly retarded fetuses, one dead fetus, and two conceptuses undergoing resorption. Increased incidences of conceptuses undergoing resorption, and retarded and dead fetuses were also observed at the 500 and 750 mg/kg/day dose levels.

Based on the results of this range-finding study, the doses summarized in Table 1 above were selected for the developmental toxicity study.

5. Dosage preparation and analysis

Test substance formulations were prepared daily immediately prior to dosing. Prior to the start of the study, the homogeneity (top, middle, and bottom) and stability of the test substance in the vehicle (water) was evaluated for a period of 4 hours. It was reported that concentration of the formulations were evaluated between the first and third day of dosing.

Results - Homogeneity and Stability Analyses: The homogeneity and stability of the test substance formulated at 12.8 and 80 g/l were 101-112% of nominal. Neither coefficients of variation nor standard deviations were presented. The storage temperature was not indicated.

Concentration Analysis: It was reported that the test formulations contained the stated amount of the test substance, but the data to support this statement were not submitted.

Data are required to confirm the nominal concentration of the administered doses.

6. Dosage administration: All doses were administered orally by gavage once daily on gestation days 6 through 18, in a volume of 5 ml/kg of body weight/day. Dosing was based on the daily body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - Mortality and clinical observations were made daily. Body weights were recorded on days 0, 6, 13, 19, and 29 of gestation and feed consumption data were recorded for gestation days 0-6, 6-13, 13-19, and 19-29. Does were sacrificed on day 29 of gestation, necropsied, and examined for gross changes especially of the uterus. The fetuses and placentae were weighed and examined for gross external abnormalities and the conceptuses undergoing resorption were measured. The following were recorded:
   - gravid uterine weight
   - number of corpora lutea
   - number of implantations
   - number of resorption sites (early or late)
   - number of live and dead fetuses

2. Fetal Evaluations: Each fetus was weighed, examined for external abnormalities and sexed. Fetuses were reared for 24 hours in an incubator at 32°C with a relative humidity of 60%. The number of fetuses that died during this period was recorded. Surviving fetuses were then sacrificed and the crown to rump length of each was measured. All fetuses were fixed in alcohol and grossly examined for visceral abnormalities. According to the study report, the brain, eyes, heart and both kidneys were removed, fixed in Bouin's fluid, cross-sectioned, and examined. The bodies were then eviscerated, cleared (aqueous KOH), and stained with Alizarin Red S in preparation for skeletal evaluation.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: Pre-implantation and post-implantation loss indices were calculated from cesarean section data. Percent fetal survival at 24 hours after delivery was also calculated. The calculations used to determine these indices were not provided.

3. Historical control data: Selected historical control data were provided. Historical control data for developmental toxicity did not include dates of collection, individual study data, litter incidence, or mean fetal incidence.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: No deaths occurred during the study. Incidences of increased water intake and reduced defecation were observed; these incidences were sporadic, not dose related, and observed in both treated and control animals and were therefore not deemed to be treatment-related.

2. Body Weight - Body weight data are summarized in Table 2. Body weight gains were not corrected for gravid uterine weights and the data presented excluded nonpregnant females, females that had aborted, and females with resorptions only. No statistically significant changes were noted in body weights or in body weight gains at any dose level. During gestation days 13-19, decreased body weight gains were noted in the 64, 160, and 400 mg/kg/day groups compared to concurrent controls. Body weight gains in the treated groups during the post-treatment interval (days 19-29) continued to be lower than the controls. Overall body weight gains (days 0-29) were decreased by 11, 31, and 23% in the low, mid- and high-dose groups. These decreases in body weight gains were not statistically different from the controls and a strict dose-dependence was not displayed; these findings were therefore considered not to be of toxicological concern.

Table 2. Maternal Mean Body Weight[a] and Body Weight Gain[b](g).

	Dose in mg/kg/day (# of Does)[c]			
Interval	Control(15)	64(14)	160(14)	400(15)
day 6 Days 0-6	2611±206 -4.6	2605±142 29.7	2541±214 9.6	2549±196 8.9
Day 13 Days 6-13	2607±200 -2.6	2610±138 -20.1	2539±208 -12.4	2540±201 -27.3
Day 19 Days 13-19	2642±197 72.6	2628±135 56.1	2544±207 22.1	2554±204 55.2
Posttreatment:Day 29 Days 19-29	2760±195 162.6	2724±152 136.9	2625±220 138.7	2651±192 140.1
Overall treatment plus Posttreatment Days 0-29	228.0	202.7	158.0	176.8

a Mean body weight data are presented in the first row of each panel. The data are excerpted from the report (p. 27) and added to this table by EPA reviewer.

b The body weight gain data are presented in the second row of each panel. The data are extracted from the study report page 28.

c Nonpregnant animals, does aborting, and those having resorptions only were excluded from the means.

3. Feed Consumption - Feed consumption data are presented in Table 3 below. A statistically significant ($p < 0.05$) reduction in feed consumption was noted in the mid- and high-dose groups during treatment. During treatment (6-13 and 13-19 days), feed consumption was reduced by 22-24% in the 160 mg/kg/day group and by 30-40% in the 400 mg/kg/day group. Feed consumption by the 64 mg/kg/day animals was slightly decreased, but the reduction was not statistically significant. The reductions in feed consumption in mid- and high dose groups were treatment-related.

Table 3. Maternal Feed Consumption (g/100 g body weight) [a].

	Dose in mg/kg/day (# of Does)[b]			
Interval	Control (15)	64 (13-14)	160 (13-14)	400 (14-14)
Pretreatment: Days 0-6	4.21±0.45	4.23±0.60	4.16±0.50	4.26±0.27
Treatment: Days 6-13	3.69±0.81	3.17±0.82	2.79±0.63*	2.20±0.59*
Treatment: Days 13-19	3.81±0.89	3.41±0.83	2.97±0.65*	2.67±1.26*
Posttreatment: Days 19-29	4.03±0.56	3.90±0.67	3.96±0.48	4.34±0.90

a Data extracted from the study report page 29. Standard deviation added by EPA reviewer.
b Nonpregnant animals, does aborting, and those having resorptions only were excluded from the means.
* $p < 0.05$.

4. Gross Pathology - No treatment-related gross pathologic findings were noted in any of the does. Missing junction of the uterus to the vagina at one side was observed in two females from the low-dose group and one female from the mid-dose group; this was not a dose-dependent finding and was not considered to be treatment-related. Except for uterine weights, which were unaffected by treatment, no organ weight data were reported.

5. Cesarean Section Data - Cesarean section observations are presented in Table 4. The numbers of corpora lutea, implantations, fetal and placental weights, and crown to rump lengths were similar between control and treated groups. In addition, the viability of the delivered fetuses during the first 24-hours after cesarean section was unaffected by treatment.

   No statistically significant differences were observed in any of the cesarean section parameters. There was one dead fetus in the 64 mg/kg/day group and two each in the 160 and 400 mg/kg/day groups, in addition, there was one abortion each in the 64 and 160 mg/kg/day groups. There were some differences in cesarean section parameters between the 160 mg/kg/day group and controls, but the differences were not dose-related and not statistically significant and were therefore considered not to be of toxicological concern.

Table 4. Cesarean Section Observations[a].

Observation	Dose (mg/kg/day)			
	0	64	160	400
# Animals Assigned (Mated)	15	15	15	15
# Animals Pregnant	15	15	15	15
Pregnancy Rate (%)	(100)	(100)	(100)	(100)
# Nonpregnant	0	0	0	0
Maternal Wastage	0	0	0	0
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	1	1	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	120	118	100	110
Corpora Lutea/Dam	8.0±1.4	8.4±1.6	7.1±1.2	7.3±1.3
Total # Implantation	95	85	85	99
Implantation/Dam	6.3±2.0	6.1±2.5	6.1±1.3	6.6±1.5
Total # Litters	15	14	14	15
Total # Live Fetuses	90	82	73	90
Live Fetuses/Dam	6.0±2.0	5.9±2.4	5.2±1.6	6.0±1.3
Total # Dead Fetuses	0	1	2	2
Dead Fetuses/Dam	0	0.07±0.27	0.14±0.36	0.13±0.35
Total # Resorptions	5	2	10	7
Early	NR	NR	NR	NR
Late	NR	NR	NR	NR
Resorptions/Dam	0.33±0.62	0.14±0.36	0.71±0.99	0.47±0.92
Early	NR	NR	NR	NR
Late	NR	NR	NR	NR
Litters with Total Resorptions	NR	NR	NR	NR
Mean Fetal Weight (g)	42.8±3.2	43.0±4.4	40.4±3.6	39.6±3.0
Males	NR	NR	NR	NR
Females	NR	NR	NR	NR
Sex Ratio (% Male)	46.67	47.56	36.99	55.56
Crown/Rump Length (mm)	99.7±3.4	99.3±4.6	97.0±4.4	96.0±3.5
Preimplantation Loss (%)	22.20	27.41	14.73	10.03
Postimplantation Loss (%)	5.94	2.98	14.89	7.98
Survival Rate at 24 Hours (%)	95.3±10.2	99.4±2.2	96.2±10.0	93.9±12.9
Placental Weight (g)	5.35±0.79	5.21±0.68	4.89±0.44	5.01±0.59

a: Data extracted from the study report pages 32 and 33. NR: Not reported.

B. DEVELOPMENTAL TOXICITY Fetal examinations included external, internal, and skeletal observations and cross-sectioning of the brain, eyes, heart and kidneys. Fetal findings were classified as major and minor defects, variations, or growth retardation. The study report did not provide an overall summary for the number of fetuses and litters affected in each evaluation category. However, summaries were provided for the incidence of normal fetuses and litters observed. Table 5 notes the most common findings.

All fetal findings were observed at incident rates that were statistically comparable to the concurrent controls with the exception of the fetal and litter incidence of a uni- or bilateral extra rib at the 13th thoracic vertebra in the 160 mg/kg/day group (fetal: 11% vs. 2.2% in controls, $p<0.05$; litter: 50% treated vs. 13.3% in controls, $p<0.05$) and the 400 mg/kg/day group (fetal: 12.2%, $p<0.01$; litter: 33.3%, not statistically significant). According to the historical control data for the performing laboratory for studies conducted between 1990 and 1993, the fetal incidence of extra ribs was within the normal range (0-11.6%) for the 160 mg/kg/day dose group and slightly above the upper limit of the normal range for the 400 mg/kg/day dose group; while the litter incidence was above the upper limit of the normal range (0-15.4%) for the 160 & 400 mg/kg/day dose groups. The mean fetal and litter incidence of 13^{th} thoracic ribs were 2.5% and 6.0%, respectively. Therefore, although the increased incidence of this variation was not strictly dose-related, the fetal incidences fell at the maximum historical incidence level and the litter incidence exceeded the historical control levels, and this variation was judged to be treatment related at 160 & 400 mg/kg/day.

There were no treatment-related defects (major or minor) or growth retardations at any dose level. However, hydrocephalus was observed in one fetus from a 400 mg/kg/day female; this finding was statistically comparable to the concurrent controls and within the range of incidence of the historical controls and was therefore considered not to be of toxicological concern.

Table 5. Summary of noteworthy fetal observations at necropsy[a]

Observations	Dose (mg/kg/day)				Historical controls [b]
	0	64	160	400	
#Fetuses (litters) examined	90 (15)	82 (14)	73 (14)	90 (15)	--
EXTERNAL/VISCERAL and ORGAN CROSS-SECTIONING					
EXTERNAL Retarded Fetuses	0 (0)	1 (1)	0 (0)	0 (0)	--
EXTERNAL/VISCERAL #Normal fetuses- (litters)	77 (15)	75 (14)	63 (14)	67 (15)	--
ORGAN CROSS-SECTION #Normal fetuses- (litters)	86 (15)	77 (14)	71 (14)	86 (15)	--
Major defects [c]:					
Head- Hydrocephalus, cranium protruding in region of parietal bone	0 (0)	0 (0)	0 (0)	1.1 (6.7)	0-9.1 (--)
Minor defects [c]:					
Eye- Blood in orbital cavity-right or bilateral	0 (0)	0 (0)	1.4 (7.1)	2.2 (13.3)	0-9.4 (--)
Thoracic Cavity- Blood in thoracic cavity	0 (0)	2.4 (14.3)	2.7 (14.3)	3.3 (13.3)	0-2.1 (--)
Thoracic Cavity/Heart/Lung- Thoracic cavity, hollow space; Heart, apex displaced; Lung, deformed lobe	0 (0)	0 (0)	0 (0)	1.1 (6.7)	0-2.2 (--)
Lung- Partially or completely fused lobes or aplasia	2.2 (6.7)	1.2 (7.1)	6.8 (21.4)	5.6 (33.3)	0-17.8 (--)
Stomach- Enlarged and taut with fluid, transverse position	7.9 (40.0)	3.7 (14.3)	4.1 (21.4)	10.0 (46.7)	0-14.7 (--)
Kidney- distended pelvis, left or bilateral	1.1 (6.7)	0 (0)	0 (0)	2.2 (6.7)	0-6.8 (--)

Observations	Dose (mg/kg/day)				Historical controls [b]
	0	64	160	400	
SKELETON					
#Normal fetuses (litters)	55 (14)	49 (13)	39 (14)	52 (15)	--
Minor defects [c]:					
Skull- unilateral or bilateral opening in parietal bone	0 (0)	3.7 (14.3)	0 (0)	0 (0)	0-8.0 (--)
Sternebra- fused	2.2 (13.3)	1.2 (7.1)	5.5 (28.6)	5.6 (26.7)	0-10.6 (--)
Retarded growth [c]:					
Skull- parietal, slight bilateral ossification	0 (0)	0 (0)	0 (0)	1.1 (6.7)	0-11.3 (--)
Caudal Vertebral Centra- < 13 centers ossified	4.4 (13.3)	12.2 (35.7)	1.4 (7.1)	3.3 (20.0)	4.3-25.6 (--)
Sternebra- unossified or partially ossified	31.1 (66.7)	32.9 (57.1)	30.1 (71.4)	22.2 (46.7)	20.5-86.7 (--)
Variation c:					
Extra rib- at 7th cervical vertebra, left or bilateral	0 (0)	0 (0)	1.4 (7.1)	2.2 (13.3)	0-12.1 (--)
at 13th thoracic vertebra, uni- or bilateral	2.2 (13.3)	0 (0)	11.0 * (50.0 *)	12.2 ** (33.3)	0-11.6 (0-15.4)

a: Data extracted from the study report pages 35-37 and 172-173.
b: Except for historical control data regarding an additional 13th thoracic rib, historical control values (1974-1993) are for the range of % fetal incidence only. For the 13th thoracic rib, historical control values were derived from studies conducted between 1990 and 1993.
c: Fetal (litter) incidence as % affected.
*: $p<0.05$
**: $p<0.01$
--: not reported

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study authors concluded that oral administration of Hoe 099730 at 160 and 400 mg/kg/day during organogenesis was associated with reduced maternal feed consumption and with statistically significant increases in the incidence of extra thoracic ribs in the offspring. Oral administration of Hoe 099730 at 64 mg/kg/day produced no maternal or developmental adverse effects.

Maternal and Developmental LOEL=160 mg/kg/day
Maternal and Developmental NOEL=64 mg/kg/day

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following oral administration of the test substance Hoe 099730 at 64, 160, or 400 mg/kg/day to pregnant rabbits on days 6-18 of gestation, minimal maternal toxicity was demonstrated by reduced feed consumption (↓22-24%; $p<0.05$) in the 160 mg/kg/day group and in the 400 mg/kg/day group (↓30-40%; $p<0.05$) during treatment days 6-13 and 13-19. Food consumption by the 64 mg/kg/day animals was slightly decreased, but the small reduction was not significantly different from the controls. The 64 mg/kg could be considered as a threshold maternal NOEL. Therefore,

   Maternal NOEL = 64 mg/kg/day
   Maternal LOEL = 160 mg/kg/day

2. DEVELOPMENTAL TOXICITY:
   a. Deaths/Resorptions: There was one dead fetus in the 64 mg/kg/day group and 2 each in the 160 and 400 mg/kg/day groups in addition, there was one abortion each in the 64 and 160 mg/kg/day groups. No statistically significant or treatment-related differences were observed in any of the cesarean section parameters.

   b. Altered Growth: There were no significant reductions or increases in fetal body weights or crown to rump lengths at any dose level.

   c. Developmental Variations: A uni- or bilateral extra rib at the 13th thoracic vertebra was observed in the 160 mg/kg/day group (fetal: 11% vs. 2.2% in controls, $p<0.05$; litter: 50% treated vs. 13.3% in controls, $p<0.05$) and the 400 mg/kg/day group (fetal: 12.2%, $p<0.01$; litter: 33.3%, not statistically significant). Although the increased incidence of this variation was not strictly dose-related, the fetal incidences fell at the maximum historical level and the litter incidence exceeded historical control level, therefore this variation was judged to be treatment-related at 160 & 400 mg/kg/day levels.

d. Malformations: There were no treatment-related developmental malformations noted at any dose level. Hydrocephalus was observed in one fetus from a 400 mg/kg/day female; this finding was statistically comparable to the concurrent controls and within the range of incidence of the historical control values and was therefore considered not to be of toxicological concern.

Based upon the increased incidence of 13th thoracic ribs, the **Developmental LOEL was 160 mg/kg/day** and **Developmental NOEL was 64 mg/kg/day.**

C. STUDY DEFICIENCIES Usually, data are required to confirm the nominal concentrations of the administered doses. Without these data, the study would have been classified as unacceptable. However, the test substance is a metabolite of glufosinate ammonium which has an adequate developmental toxicity data base. In addtion, this study was submitted for verification of the NOEL and LEL provided by the registrant to show that toxicity of various metabolites is less than that of the parent compound. Under the circumstance, this study is considered as **acceptable/nonguideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in rabbits.

012647

DATA EVALUATION RECORD

GLUFOSINATE - AMMONIUM

Study Type: §83-3(b); Testing of Hoe 061517:
For Embryotoxicity in the Himalayan Rabbit After Oral Administration

Work Assignment No. 2-37A (MRID 44076210)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Sandra Daussin

Signature: Sandra Daussin
Date: 12/3/96

Secondary Reviewer:
Michael Norvell, Ph.D.

Signature: Michael Norvell
Date: 12/12/96

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 1/7/97

Quality Assurance:
William Spangler, Ph.D.

Signature: William J Spangler
Date: 01/09/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: Whang Phang, Ph.D. 2/12/98
Review Section III, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Susan L. Makris, M.S. 6/1/98
Review Section III, Toxicology Branch II (7509C)

012647

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rabbit
OPPTS Number: 870.3700 OPP Guideline Number: §83-3(b)

DP BARCODE: D229929 SUBMISSION CODE: S509558
P.C. CODE: 128850 TOX. CHEM. NO.: 580I

TEST MATERIAL (PURITY): Hoe 061517 (99.6%)(a metabolite of glufosinate ammonium)

SYNONYMS: 3-methylphosphinoco-propionic acid

CITATION: Albrecht, M. and Baeder, C. (1994) Testing of Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC99 0003) For Embryotoxicity in the Himalayan Rabbit After Oral Administration. Hoechst Aktiengesellschaft, 6230 Frankfurt am Main 80, Germany. Laboratory Study Numbers, RK0546 & 87.0727; Report Number A52160, February 2, 1994. MRID 44076210. Unpublished.

SPONSOR: AgrEvo USA Company, Little Falls Centre One, 2711 Centerville Road, Wilmington, DE

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44076210) Hoe 061517 (a metabolite of glufosinate ammonium) (99.6% a.i.) in distilled water was administered to 15 Hoe:HIMK (SPFWiga) Himalayan rabbits/dose/group by gavage at dose levels of 0, 50, 100, or 200 mg/kg/day from days 6 through 18 of gestation.

Maternal toxicity was demonstrated at 100 mg/kg/day, as a dose-related increase in abortions (7% vs. 0% in controls) and mortality (7% vs. 0% in controls), clinical signs (disequilibrium), and reductions in food and water consumption, body weight gain, and fecal output. At 200 mg/kg/day, maternal toxicity was demonstrated by treatment-related clinical signs of toxicity (disequilibrium, and/or straddled fore-limbs), increases in abortions (27% vs. 0% in controls) and mortality (33% vs. 0% in controls), reductions in body weight gain, food and water consumption, and fecal output. In addition, treatment-related gross pathology was noted in the kidneys of the high-dose animals and was characterized as uneven, rough surface of one high-dose

dam, and light-brown coloring of the renal cortex of three of the four aborting high-dose dams. Corroborative treatment-related increases in the mean kidney weights was also noted at 200 mg/kg/day.

At 50 mg/kg level, no treatment-related deaths or effects were reported. **The maternal LOEL is 100 mg/kg/day, based on increased abortions and mortality and reductions in food and water consumption, body weight gain, and fecal output. The maternal NOEL is 50 mg/kg/day.**

There were no treatment-related effects noted in developmental parameters at any dose level. **A developmental LOEL was not observed (>200 mg/kg/day). The developmental NOEL is 200 mg/kg/day.**

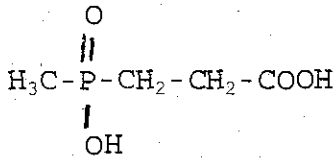
This developmental toxicity study in the rabbit is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in the rabbit. In order to upgrade the study, the sponsor must submit data confirming the nominal concentrations of the administered doses and the stability of the test substance in distilled water.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Hoe 061517; 3-methylphosphinoco-propionic acid (a metabolite of glufosinate ammoniun)
   Description: Technical, white powder
   Lot/Batch #: H 404
   Purity: 99.6% a.i.
   CAS #: 77182-82-2
   Structure:



2. Vehicle: Distilled water

3. Test animals: Species: Himalayan rabbit
Strain: Hoe:HIMK (SPFWiga)
Age at mating: 7-8 months
Weight at mating: 2653 ± 164 g
Source: Hoechst breeding colony, Frankfurt, Germany
Housing: Individually (except during mating) in metal-barred cages with wire-mesh floors
Diet: ERKA 6000 standard diet, ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 21.0 - 22.5 C
Humidity: 38 - 67%
Air changes: 16-20/hr
Photoperiod: 14 hrs dark/10 hrs light
Acclimation period (P): At least 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 8/11/88 End: 10/26/88

2. Mating: Females in estrous were paired on a 1:1 basis with stock males of the same strain. Animals with sperm in the vaginal smear were mated again after 6 hours to assure that a successful mating occurred. Day 0 of gestation was designated as the day of mating.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	15
Low (LDT)	50	15
Mid (MDT)	100	15
High (HDT)	200	15

4. Dose selection rationale: In a range-finding study summarized in the current submission, Hoe 061517 (% a.i. not indicated) was administered orally to 2 pregnant rabbits/dose at dosages of 0, 20, 40, 160, 250, 400, or 1250 mg/kg/day on days 6 to 18 of gestation. Dams were sacrificed on day 29 of gestation.

There were no treatment-related findings in either the dams or the fetuses at doses ≤160 mg/kg/day. At 250 mg/kg/day, one dam was found dead on day 13 of gestation following flabbiness, disequilibrium, marked anorexia, and weight loss. The other dam dosed at 250 mg/kg/day lost weight during the first week of treatment but delivered normally developed live fetuses. At 400 mg/kg/day one dam was sacrificed moribund on day 10 of gestation, and the other dam was weak, showed signs of disequilibrium, and aborted by day 10 of gestation. At 1250 mg/kg/day, both dams were found dead on day 8 of gestation.

Based upon these results, the subsequent full developmental toxicity study in rabbits was conducted at dosages of 0, 50, 100, or 200 mg/kg/day.

5. Dosage preparation and analysis: Test substance formulations were prepared daily by mixing appropriate amounts of test substance with distilled water and were administered within 3 hours of preparation. Prior to the start of the study, stability of the test substance in water was evaluated for a period of 5 hours at an unspecified temperature. Concentrations of the formulations were not evaluated or were not reported.

   Results - Stability Analysis: The study report states that the stability of the solutions "was guaranteed for 5 hours after preparation (Analytical Laboratory Hoest AG, statement of 9/7/88)."

   Data are required to confirm the nominal concentration of the administered doses and the stability of the test substance in distilled water.

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 6 through 18, in a volume of 5 ml/kg of body weight/day. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality and clinical signs daily. Body weight data were recorded on gestation days 0, 6, 13, 19, and 29 and food consumption data were recorded for gestation days 0-6, 6-13, 13-19, and 19-29. Dams were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of a gross exam of the thoracic and abdominal cavities and the following organs were weighed: heart, liver, kidneys, and spleen. The reproductive tract was removed and the

following were recorded:
- number of implantation sites
- number of live and dead fetuses
- number of resorptions (early and late)
- number of corpora lutea in each ovary
- placental weights

2. Fetal Evaluations - Each fetus was weighed, examined for signs of life and external abnormalities and the sex was recorded. Fetuses were reared for 24 hours in an incubator at 32°C with a relative humidity of 60%. The number of fetuses that died during this period was recorded. Surviving fetuses were then sacrificed and the crown to rump length was determined. All fetuses were fixed in alcohol and a gross exam of the viscera was performed. According to the report, brain, eyes, heart and both kidneys were removed, fixed in Bouin's fluid, cross-sectioned, and examined. The bodies were then eviscerated, cleared (aqueous KOH), and stained with Alizarin Red S to examine for skeletal alterations.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: Preimplantation and postimplantation loss indices were calculated from cesarean section records of animals in the study. Percent fetal survival at 24 hours after delivery was also calculated. Formulas used for their calculation were not provided.

3. Historical control data: Selected historical data were provided. Historical data for developmental toxicity did not include dates of collection, individual study data, litter incidence, or mean fetal incidence.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: At 100 mg/kg/day, one dam was found dead on day 24 of gestation and one dam was sacrificed on day 22 after hemorrhaging vaginally and aborting. The dam that died was flabby for 7 days prior to death and the dam that aborted exhibited disequilibrium in the 2 days prior to aborting. As a reflection of reduced food consumption, fecal output in these animals was also decreased. The abortion, death, and clinical signs

(flabbiness or disequilibrium) in these animals were treatment-related.

At 200 mg/kg/day, five dams were found dead on days 18-20 of gestation. Four additional high-dose dams were sacrificed on days 16, 17, or 25 after hemorrhaging vaginally and aborting. Treatment-related clinical signs that were noted in the high-dose animals that died or aborted included flabbiness and/or disequilibrium. These observations were persistent, lasting between 1 and 7 days and first appeared between days 12 to 18 of gestation. In addition, one of the high-dose dams that died exhibited straddled fore-limbs. Fecal output was also decreased in the high-dose animals that died or aborted.

In addition, one dam at 200 mg/kg/day aborted on day 3 of gestation (prior to treatment), and one dam at 50 mg/kg/day died from faulty intubation. However, these animals were replaced and were not evaluated further in the study.

No treatment-related clinical findings or deaths were observed at 50 mg/kg/day.

2. Body Weight - Mean body weights and body weight gain data are summarized in Table 2. Body weight gains were not corrected for gravid uterine weights and the data presented only include pregnant animals that survived until the day 29 sacrifice. For these animals, body weight gains were comparable to the controls at all dose levels. For the dams that died and/or aborted in the mid- and high-dose groups, body weight gains were either stagnated or the animals experienced a weight loss during treatment.

Table 2. Maternal body weight and body weight gain (g)

Interval	Dose in mg/kg/day (# of Dams)[a]			
	Control (15)	50 (15)	100 (13)	200 (6)
Pretreatment: Days 0-6	2661±155[b] 21.2[c]	2683±166 41.1	2633±134 24.9	2668±203 43.3
Treatment: Days 6-13	2679±149 14.7	2701±151 -3.8	2649±123 7.4	2680±183 -19.3
Treatment: Days 13-19	2716±143 58.1	2738±114 76.7	2683±116 60.5	2695±193 48.3
Posttreatment: Days 19-29	2817±166 144.8	2840±116 127.6	2785±124 144.5	2799±215 160.3

a Nonpregnant animals, dams aborting, and those not surviving to Day 29 were excluded from the means.
b Mean body weight data excerpted from the report (p. 36) MRID 44076210.
c mean Body weight gain data excerpted from the report (p. 37) MRID 44076210.

4-

3. Food Consumption - Food consumption data for does that survived to cesarean section are summarized in Table 3. Food consumption for the mid- and high-dose dams that died and/or aborted (not included in Table 3) was markedly decreased. These dams typically consumed between 0 and 19 g of food/day in the prior week to the abortion and/or death whereas control animals consumed weekly averages of 87-96 g/day. Water consumption was also decreased in these animals. The data presented in Table 3 do not include dams that died or aborted prior to day 29. Food consumption was comparable to the controls throughout the study at all dose levels for dams that survived to the end of the study and were cesarean sectioned on gestation day 29.

Table 3. Maternal food consumption (g/100 g body weight)[a]

Interval	Dose in mg/kg/day (# of Dams)[b]			
	Control (15)	50 (15)	100 (13)	200 (6)
Pretreatment: Days 0-6	3.40	3.62	3.53	4.18
Treatment: Days 6-13	3.56	3.35	3.32	3.01
Treatment: Days 13-19	3.22	3.14	3.26	3.21
Posttreatment: Days 19-29	3.42	3.16	3.28	3.60

a Data extracted from the study report page 35 (MRID 44076210).
b Nonpregnant animals, dams that aborted, and those not surviving to Day 29 were excluded from the means.

4. Gross Pathology - A gross necropsy was performed and organ weights were determined for all dams in the study except the one dam at 100 mg/kg/day and four of the five dams at 200 mg/kg/day that were found dead. These animals died in the night and a significant degree of general autolysis occurred before a necropsy could be performed. Of the animals examined, gross pathologic lesions noted in the kidneys included light grey retractions on the surface in two controls and one mid-dose dam, uneven, rough surface of one high-dose dam, and light-brown coloring of the renal cortex of three of the four aborting high-dose dams. Other gross findings at 200 mg/kg/day included brown fluid in the vagina and uterus of one dam that aborted and numerous blackish follicles on both ovaries of this same dam.

At 200 mg/kg/day, the mean combined absolute kidney weight

was increased compared to the controls, although the difference was not statistically significant (15.35 g vs. 14.22 g). The increase in the mean kidney weight was primarily due to the relatively high kidney weights of four high-dose dams that were either found dead or aborted (23.5-31.5 g vs. 12.8-20.0 g for the remaining high-dose dams). The mean absolute liver weight of the high-dose dams was also slightly increased compared to the controls (55.8 g vs. 53.7 g) but was not statistically significant. This increase was due to one high-dose dam that was found dead with a liver weight of 107.8 g (liver weights of the remaining high-dose dams were 48.3-79.5 g). The increase in mean liver weight was considered treatment-related.

The study author concluded that the findings in the kidney were not treatment-related at any dose level because similar findings have been observed in the historical controls; however, historical control data to support this assertion were not provided. As renal macroscopic pathology was noted in the animals that aborted and corroborative increases in kidney weights were observed in these same animals, it appears that the kidney is a target organ. Therefore, gross renal pathology and increases in kidneys weights in the high-dose group are considered treatment-related findings.

Heart and spleen weights were comparable to controls at all dose levels and kidney and liver weights were comparable to the controls at the low- and mid-dose levels.

5. Cesarean Section Data - Cesarean section observations are presented in Table 4. The numbers of corpora lutea, implantations, the extent of pre-implantation loss, fetal and placental weights, crown to rump lengths, and the percent males were statistically similar between control and treated groups. In addition, the viability of the delivered fetuses during the first 24-hours after c-section was unaffected by treatment.

Viability as expressed as the number of live fetuses/dam was comparable between the treatment groups and the controls. In the high dose-group, post implantation loss was increased (24.7 vs 18.8 in controls), and the mean number of resorptions/dam was increased also (2.17 vs. 1.13 in controls). The increase in resorptions was not significantly different from the controls. The increase was due to resorptions occurring in three of the six litters examined at cesarean section. The number (percent) of resorptions for each litter were: 9(90.0), 1(25.0), and 3(33.0).

In historical control data for New Zealand white rabbits on study from 1992-1994, published by the Middle Atlantic Reproduction and Teratology Association (MARTA) and the

Midwest Teratology Association (MTA) in 1996, a review of 130 studies demonstrated a mean (±SD) resorption rate of 0.55±0.36 resorption/pregnant femlae or 7.02±5.69%. The resorption rate in the rabbits treated with 200 mg/kg/day of Hoe 061517 exceeded that of the historical controls. It is also noted that the concurrent control resorption rate values for the the study on Hoe 061517 exceeded historical control values, suggesting that the high rate of resorptions in the control does was responsible for the lack of statistical significance for this parameter at the high-dose.

The study authors concluded that this was an indication of a treatment-related increase in the intrauterine death rate at the high-dose. Tox. Branch II agrees with this assessment. The historical control data provided were insufficient and not in an appropriate format for comparing resorptions/dam in this study. The historical data indicated percent of early and late intrauterine deaths and not resorptions/dam.

In support of the conclusion that treatment-related postimplantation loss was occurring at the high dose, a treatment-related increase in abortions was noted in the high- and mid-dose dams and embryotoxicity was noted in most of these dams. At the mid-dose, one dam aborted on day 22. One fetus in this dam was alive and the other seven were undergoing resorption at the time of abortion. At the high-dose, four dams aborted on days 16, 17, or 25. One of the aborting high-dose dams had nine normal conceptuses. The remaining three high-dose dams had 5-8 conceptuses that were all undergoing resorptions.

Additionally, for one of the five high-dose dams and the one mid-dose dam that were found dead, the conceptuses were normal. Of the remaining four high-dose dams that were found dead, the conceptuses were either undergoing resorptions or were severely stunted (severe embryotoxicity).

Table 4. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	50	100	200
# Animals Assigned (Mated)	15	15	15	15
# Animals Pregnant Pregnancy Rate (%)	15 (100)	15 (100)	15 (100)	15 (100)
# Nonpregnant	0	0	0	0
Maternal Wastage				
# Died	0	0	1	5
# Died Pregnant	0	0	1	5
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	1	4
# Premature Delivery	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	118 7.9±1.2	126 8.4±1.3	101 7.8±1.2	48 8.0±1.8
Total # Implantations Implantations/Dam	96 6.4±1.8	104 6.9±1.5	81 6.2±1.6	45 7.5±2.2
Total # Litters	15	15	13	6
Total # Live Fetuses Live Fetuses/Dam	79 5.3±2.0	95 6.3±1.5	72 5.5±1.7	32 5.3±2.8
Total # Dead Fetuses Dead Fetuses/Dam	not reported	not reported	not reported	not reported
Total # Resorptions	17	9	9	13
Early	14	7	7	11
Late	3	2	2	2
Resorptions/Dam	1.13±1.06	0.60±1.06	0.69±0.85	2.17±3.54
Early	0.93±1.10	0.47±0.64	0.54±0.52	1.83±3.54
Late	0.20±0.41	0.13±0.52	0.15±0.55	0.33±0.82
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g) b	42.6±2.7	41.4±3.2	42.1±1.8	40.5±2.0
Crown/Rump Length (mm) b	97.1±2.6	97.1±2.4	96.7±2.0	96.0±3.9
Sex Ratio (% Male)	57.0	53.7	51.4	50.0
Preimplantation Loss (%)	19.0	17.3	20.1	7.22
Postimplantation Loss (%)	18.8	8.08	11.0	24.7
Survival rate at 24 hours (%)	94.7	97.4	98.5	100

a Data extracted from the study report pages 38 and 39 (MRID 44076210).
b Data for fetal weights and crown/rump lengths were not presented for each sex separately.

B. DEVELOPMENTAL TOXICITY

1. External, Visceral, and Skeletal Examinations: Fetal examinations included external, internal, and skeletal observations at necropsy and cross-sectioning of the brain, eyes, heart and kidneys. Fetal findings were classified as malformations, minor anomalies, variations, or growth retardation. The study report did not provide an overall

summary for the number of fetuses and litters affected in each evaluation category. However, summaries were provided for the incidence of normal fetuses and litters observed. Table 5 notes the most common findings.

There were no treatment-related malformations, minor anomalies, variations, or growth retardation at any dose level. All fetal findings were observed at incident rates that were statistically comparable to the concurrent controls with the exception of an increased fetal and litter incidence of retarded growth (ossification of less than 13 centers) in the caudal vertebrae of the low dose group (fetal: 24.2% vs. 7.6% in controls, $p<0.05$; litter: 73.3% vs. 33.3% in controls, $p<0.05$). However, this is not considered a treatment-related finding as it was not dose-related.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS The study report concluded that oral administration of Hoe 061517 at 100 and 200 mg/kg/day to pregnant rabbits during organogenesis was associated with treatment-related increases in maternal mortality, abortions, and intrauterine deaths. The maternal deaths and abortions were preceded by treatment-related clinical signs of intolerance (disequilibrium and/or flabbiness), reduced food and water consumption, and body weight reductions. No teratogenic effects were noted at any dose level. Oral administration of Hoe 061517 at 50 mg/kg/day produced no maternal or developmental adverse effects.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following oral administration of Hoe 061517 (a metabolite of glufosinate ammonium, 99.6% a.i.) at a dose of 100 mg/kg/day, maternal toxicity was demonstrated by dose-related increases in abortions and mortality, clinical signs of toxicity, and reductions in food and water consumption, body weight gain, and fecal output. One dam was found dead on day 24 of gestation and one dam was sacrificed on day 22 after hemorrhaging vaginally and aborting. The dam that died was flabby for 7 days prior to death and the dam that aborted exhibited disequilibrium in the 2 days prior to aborting. Food and water consumption, and body weight gains for pregnant animals that survived until the day 29 sacrifice were comparable to those in the control group and at all other dose levels. However, for the mid-dose animals that died or aborted, food and water consumption and fecal output were reduced and both animals lost weight in the week prior to sacrifice/death.

Table 5. Summary of noteworthy fetal observations at necropsy[a]

Observations	Dose (mg/kg/day)				Historical controls [b]
	0	50	100	200	
#Fetuses (litters) examined	79 (15)	95 (15)	72 (13)	32 (6)	---
EXTERNAL/VISCERAL and ORGAN CROSS-SECTIONING					
EXTERNAL/VISCERAL #Normal fetuses- (litters)	74 (15)	88 (15)	71 (13)	29 (6)	---
ORGAN CROSS-SECTION #Normal fetuses- (litters)	78 (15)	93 (15)	71 (13)	32 (6)	---
Minor anomalies [c]:					
Lung-Partially or completely fused lobes	0 (0)	3.2 (20.0)	0 (0)	3.1 (16.7)	---
Stomach-enlarged and taut with fluid, transverse position	3.8 (20.0)	3.2 (20.0)	1.4 (7.7)	6.3 (33.3)	---
SKELETON					
#Normal fetuses litters	26(33)[d] 11(73)	27(28) 12(80)	28(39) 10(77)	13(41) 6(100)	---
Major anomalies [c]:					
Scoliosis in region of thoracic vertebra, dysplastic and fused 11th and 12th thoracic vertebral centra, anaplastic 12th thoracic vertebral arch and rib	0 (0)	1.1 (6.7)	0 (0)	0 (0)	0-2.3 (---)
Minor anomalies [c]:					
Skull-unilateral opening in parietal bone	3.8 (20.0)	0 (0)	0 (0)	3.1 (16.7)	---
Caudal vertebral centra-dislocated, fused, longitudinally displaced	3.8 (20.0)	3.2 (20.0)	1.4 (7.7)	3.1 (16.7)	---
Sternebra- longitudinally displaced, fused, dysplasia	5.1 (20.0)	2.1 (13.3)	5.6 (23.1)	6.3 (16.7)	---
Retarded growth [c]:					
Caudal vertebra- <13 centers ossified	7.6 (33.3)	24.2* (73.3*)	8.3 (30.8)	12.5 (50.0)	10.2-22.4 (---)
Sternebra- unossified or partially ossified	54.4 (80.0)	55.8 (93.3)	54.2 (100)	40.6 (83.3)	---

a Data extracted from the study report pages 40-42 (MRID 44076210).
b Historical control values are for the range of % fetal incidence only and were given only for those findings shown.
c Percent affected [Fetal (litter) incidences]
d Percentage
* Statistically significant, $p<0.05$.

At 200 mg/kg/day, maternal toxicity was demonstrated by treatment-related clinical signs of toxicity, increases in abortions and mortality, reductions in body weight gain, reduced food and water consumption, reduced fecal output, increased organ weights, and gross pathology. Five dams were found dead on days 18-20 of gestation. Four additional high-dose dams were sacrificed on days 16, 17, or 25 after hemorrhaging vaginally and aborting. Treatment-related clinical signs of toxicity that were noted in the high-dose animals that died or aborted included flabbiness and/or disequilibrium. These observations were persistent, lasting between 1 and 7 days and first appeared between days 12 to 18 of gestation. In addition, one of the high-dose dams that died exhibited straddled fore-limbs. For the high-dose dams that died or aborted, food and water consumption and fecal output were reduced and body weight gains were either stagnated or the animals experienced a weight loss during treatment.

Treatment-related gross pathologic lesions seen in the kidneys of the high-dose animals were described as uneven, rough surface of one high-dose dam, and light-brown coloring of the renal cortex of three of the four aborting high-dose dams. In addition, a treatment-related increase in mean combined kidney weights at 200 mg/kg/day was noted, although the difference from the controls was not statistically significant (15.35 g vs. 14.22 g). This increase was primarily due to the relatively high kidney weight values of four high-dose dams that were either found dead or aborted (23.5-31.5 g vs. 12.8-20.0 g for the remaining high-dose dams). The study author concluded that the gross findings in the kidney were not treatment-related at any dose level because similar findings have been observed in the historical controls. The reviewer disagrees with this conclusion. As renal marcroscopic pathology was noted in the animals that aborted and corroborative increases in kidney weights were observed in these same animals, it appears that the kidney is a target organ of Hoe 061517.

A treatment-related increase in mean liver weight was also noted at 200 mg/kg/day, although the difference from the controls was not statistically significant (55.8 g vs. 53.7 g). This increase was due to one high-dose dam that was found dead with a liver weight of 107.8 g (liver weights of the remaining high-dose dams were 48.3-79.5 g).

There were no treatment-related deaths, clinical signs of toxicity or gross pathologic findings, changes in body weight gains, food consumption, or organ weights observed in rabbits at the 50 mg/kg/day dose level.

Maternal NOEL = 50 mg/kg/day
Maternal LOEL = 100 mg/kg/day (based on increase in the incidence of abortion, mortality, clinical signs of toxicity, decreased body weight gain, and reduced food and water consumption)

2. DEVELOPMENTAL TOXICITY:

The numbers of corpora lutea, implantations, the extent of pre-and post-implantation losses, fetal and placental weights, crown to rump lengths, and the percent males were similar between control and treated groups. In addition, the viability of the delivered fetuses during the first 24-hours after c-section was unaffected by treatment.at any dose level.

In the high-dose group, there was an increase in post implantation loss (25% vs. 19% in controls), and the mean number of resorptions/dam was also increased (2.2% vs. 1.1% in controls). It is not possible to determine the precise extent to which maternal toxicity was a contributing factor in the intrauterine fetal deaths note at the mid- and high-dose levels (100 and 200 mg/kg/day, respectively). However, because the incidence of postimplantation loss in mid-dose does that survived to cesarean section was less than concurrent and historical controls (MARTA/MTA) values, it is suggested that those intrauterine deaths observed in aborting or moribound does at that dose level were attributed primarily to maternal toxicity. **The mid-dose (100 mg/kg/day), therefore, is established as the developmental NOEL.** At the high-dose (200 mg/kg/day), where postimplantation loss was increased in does that survived to cesarean section, maternal toxicity did not appeared to be the primary contributor to intrauterine death. **The developmental LOEL is, therefore, established at 200 mg/kg/day, based on increased postimplantation loss.**

C. STUDY DEFICIENCIES: This study is not required by the Agency. It is submitted to the Agency for the purpose of verifying the values of LEL and NOEL previously presented to the agency to show that the metabolites of glufosinate ammonium are less toxic than the parent compound.

A major deficiency was that concentrations of the administered formulations were not evaluated or were not reported. Stability data also were not provided although the study report states that the stability of the solutions "was guaranteed for 5 hours after preparation (Analytical Laboratory Hoest AG, statement of 9/7/88)". Without these

data, the developmental study is classified as **Unacceptable/Guideline** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(b)) in rabbits. To upgrade the study the sponsor must submit data confirming the nominal concentrations of the administered doses and the stability of the test substance in distilled water.

Another deficiency was that historical control data provided for resorptions/intrauterine deaths were not in a useful format for comparison with the study report. The historical data indicate percent of early and late intrauterine deaths, however, it is not clear how these values were calculated and individual study data were not provided. In addition, historical control data provided for morphological findings in the fetuses were incomplete and were not in a useful format for comparison with the study data. The historical control data did not include dates of collection, individual study data, litter incidence, or mean fetal incidence. However, as the developmental alterations found were comparable to the concurrent controls or lacked a dose-response and the treatment-related effect noted on resorptions at the high-dose was not the sole end point, these deficiencies did not affect the adequacy of the study to determine developmental or maternal toxicity.

Mean fetal data such as body weight and crown to rump length should be provided for males, females, and combined sexes. If the mean of one sex is statistically significant, it may indicate a developmental effect.

In addition, gravid uterine weights were not presented. However, this is a minor deficiency and does not affect the adequacy of the study to determine developmental and maternal toxicity.

DATA EVALUATION RECORD 012647

GLUFOSINATE AMMONIUM

Study Type: 83-3(a); Testing of Hoe 061517 - Substance Technical for Embryotoxicity in the Wistar Rat After Oral Administration

Registration Application and Tolerance Petition

Work Assignment No. 1-37L (MRID 44076209)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Sandra Daussin

Signature: Sandra Daussin
Date: 12/3/96

Secondary Reviewer:
Michael Norvell, Ph.D.

Signature: Michael Norvell
Date: 12/5/96

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 12/10/96

Quality Assurance:
William Spangler, Ph.D.

Signature: William Spangler
Date: 12/10/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: Whang Phang, Ph.D. 6/3/98
Reregistration Branch I/HED (7509C)

EPA Secondary Reviewer: Susan Makris, M.S. 6/3/98
Toxicology Branch I/HED (7509C)

012647

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rat
(§83-3(a); OPPTS, 870.3700)

DP BARCODE:	D229929	SUBMISSION CODE:	S509558
P.C. CODE:	128850	TOX. CHEM. NO.:	580I
MRID	44076209		

TEST MATERIAL (PURITY): Hoe 061517 (a metabolite of glufosinate ammonium)(99.6% a.i.)

SYNONYMS: 3-methylphosphinico-propionic acid

CITATION: Albrecht, M. and Baeder, C., (1994) Testing of Hoe 061517 - Substance Technical (Code: Hoe 061517 0Q ZC 99 0003) for Embryotoxicity in the Wistar Rat After Oral Administration. Pharma Research Toxicology and Pathology, Germany. Study Numbers: RR0545 & 87.0726; Report Number A52161; February 3, 1994. MRID 44076209. Unpublished.

SPONSOR: AgrEvo USA Company, 2711 Centerville Road, Wilmington, DE

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44076209) Hoe 061517 (a metabolite of glufosinate ammonium; 99.6% a.i.) in distilled water was administered by gavage to 20 presumed pregnant Wistar rats/dose at dose levels of 0, 100, 300, or 900 mg/kg/day from days 6 through 17 of gestation

Maternal toxicity was demonstrated at 900 mg/kg/day, maternal toxicity was demonstrated by one death, treatment-related clinical findings (persistent piloerection and/or increased urinary output), increased absolute kidney weights. **The maternal LOEL is 900 mg/kg/day; NOEL, 300 mg/kg/day.**

At 900 mg/kg/day, increases in the incidence of total litter loss and in the fetal and litter incidence of wavy and/or thickened ribs were found. There were additional treatment-related effects noted in developmental parameters. **Therefore, the developmental LOEL for developmental toxicity is 900 mg/kg/day; NOEL, 300 mg/kg/day.**

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The developmental toxicity study in the rat is classified as **Acceptable/non-guideline** for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat. It should be noted that this study was submitted for verification of the NOEL and LOEL provided by the registrant to show that the toxicity of various metabolites is less than that of the parent compound.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Hoe 061517 (glufosinate ammonium metabolite)
   Description: Technical, white powder
   Lot/Batch #: H 404
   Purity: 99.6% a.i.
   CAS #: 77182-82-2

2. Vehicle: distilled water

3. Test animals: Species: Rats
   Strain: Hoe:WISKf(SPF71), Wistar rats
   Age at mating: 10-11 wks
   Weight at mating: 189 ± 9 g
   Source: Hoechst breeding colony, Frankfurt, Germany
   Housing: Individually (except during mating) in plastic cages
   Diet: Altromin Breeding Diet TPR® 1310 for Rats and Mice, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   - Temperature: 22 - 23 C
   - Humidity: 44 - 60%
   - Air changes: 16-20/hr
   - Photoperiod: 12 hrs dark/12 hrs light

   Acclimation period (P): At least 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 4/25/88 end: 6/15/88

2. Mating: Females in estrous were paired on a 1:1 basis with stock males of the same strain. Day 1 of gestation was designated as the day sperm were found in a vaginal smear.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	20
Low (LDT)	100	20
Mid (MDT)	300	20
High (HDT)	900	20

4. Dose selection rationale: In a range-finding study summarized in the current submission, Hoe 061517 (% a.i. not indicated) was administered orally to 3 pregnant rats/dose at dosages of 0, 10, 50, 250, 500, or 1,250 mg/kg/day on days 7 to 16 of gestation. Dams were sacrificed on day 21 of gestation.

There were no treatment-related findings in either the dams or the fetuses at doses ≤500 mg/kg/day. At 1,250 mg/kg/day, piloerection was observed in all three dams after the third treatment. One high-dose dam died on day 10 of gestation following marked anorexia and weight loss. Vaginal hemorrhaging and decreases in feed consumption and body weight were observed in one high-dose dam. One fetus of this dam was normal, the remaining were either undergoing resorption, malformed, or had growth retarded. One high-dose dam delivered normal fetuses.

Based upon these results, the subsequent full developmental toxicity study in rats was conducted at dosages of 0, 100, 300, or 900 mg/kg/day.

5. Dosage preparation and analysis Test substance formulations were prepared daily by mixing appropriate amounts of test substance with distilled water and were administered within 3 hours of preparation. Prior to the start of the study, stability of the test substance in water was evaluated for a period of 5 hours at an unspecified temperature. Concentration and homogeneity (top, middle, and bottom) of the formulation were not evaluated or were not reported.

Results - Stability Analysis: The study report states that the stability of the solutions "was guaranteed for 5 hours after preparation (Analytical Laboratory Hoechst AG, statement of 9/7/88)".

Data are required to confirm the nominal concentration and homogeneity of the administered doses and the stability of the test substance in distilled water

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 7 through 16, in a volume of 5 ml/kg of body weight/day. Dosing

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was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality or clinical signs of toxicity daily. Body weight data were recorded on gestation days 0, 7, 14, 17, and 21 and feed consumption data were recorded for gestation days 1-7, 7-14, 14-17, and 17-21. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of gross evaluations of the thoracic and abdominal cavities and the following organs were weighed: heart, liver, kidneys, and spleen. The reproductive tract was removed and the following data were recorded:
   - number of implantation sites
   - numbers of live and dead fetuses
   - numbers of resorptions (early and late)
   - number of corpora lutea in each ovary
   - placental weights

2. Fetal Evaluations - Each fetus was weighed, examined for external abnormalities and the crown to rump length and sex was recorded. For each litter, approximately half of the fetuses and all fetuses found dead were fixed in alcohol, cleared (aqueous KOH), and stained with Alizarin Red S to examine for skeletal alterations. The remaining fetuses were fixed in Bouin's fluid and the bodies were cross-sectioned to examined for soft tissue alterations.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures

2. Indices: Preimplantation and postimplantation loss indices were calculated from cesarean section records, of animals in the study. Formulas used in these calculations were not provided.

3. Historical control data: For the developmental toxicity, historical control data were not useful in the format provided. Historical control data did not include dates of collection, individual studies data, litter incidences, or mean fetal incidences.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: One of the high-dose dams was found dead on day 11 of gestation, and a significant degree of general autolysis occurred. The cause of death was not given. However, on day 10 of gestation, this dam exhibited "flabbiness", disequilibrium, piloerection, and increased urinary excretion. Two additional dams in the 900 mg/kg/day dose group were sacrificed moribund on gestational days 7 and 13, respectively; the cause of death of these animals was reported to be faulty intubation. There were no treatment-related deaths in the low and mid-dose group rats

   Two high-dose dams were reported to have bloody secretions in the vagina on gestational days 13 or 15, and both dams were found to have total litter loss. Treatment-related clinical findings in high-dose dams included persistent piloerection in 10/16 dams and/or increased urinary output in 8/16 dams. These observations were persistent, lasting between 1 and 6 days and first appeared between gestational days 8 and 15. Corroborative increases in kidney weights were observed in the high-dose dams.

   No treatment-related clinical findings were observed at the low and mid-dose levels. Although alopecia was observed at a low incidence in all dose groups including the controls, it was attributed to the hereditary predisposition of this strain of rats.

2. Body Weight - The mean body weight and body weight gain data are summarized in Table 2. Body weight gains were not corrected for gravid uterine weights, and the data presented only included pregnant animals that survived until the terminal sacrifice (day 21). Body weight gains were comparable to the controls at the low and mid-dose levels.

   At 900 mg/kg/day, body weight gains were statistically significantly decreased by 41% ($p < 0.05$) during the first week of treatment. The study author indicated that this initial decrease in body weight gains was treatment-related. Body weight gains were only slightly decreased (3%) on days 14-17 and were increased in the post-treatment period (7%); these differences were not statistically significant.

Table 2. Maternal mean body weight[a] and body weight gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)			
	Control (20)	100 (20)	300 (19)	900 (16)
Pretreatment: Day 7	205±10[a]	202±10	200±8	207±10
Days 0-7	29.0[b]	30.9	26.9	29.5
Treatment: Day 14	236±12	233±12	229±12	231±12
Days 7-14	32.3	31.3	30.0	18.9*
Treatment: Day 17	261±13	259±15	253±14	250±17
Days 14-17	18.6	18.9	19.3	18.1
Posttreatment: Day 21	295±12	292±18	289±17	285±21
Days 17-21	48.5	47.3	51.9	51.8

a Mean body weight excerpted from the report (p. 34) by EPA reviewer.
b Data extracted from the study report page 35; the body weight gains of the non-pregnant dams were not included in these data.
* Statistically significant ($p<0.05$).

3. Feed Consumption - Feed consumption data were expressed as g/100 g body weight are summarized in Table 3. For the low and mid-dose groups, feed consumption was comparable to the controls throughout the study

At the high-dose, feed consumption was decreased (↓22%, $p<0.05$) during the first week of treatment with a subsequent increase afterward (↑3 and 10%, $p<0.05$, days 14-17 and 17-21, respectively). This fluctuation in feed consumption was considered to be a treatment-related effect by the study authors. In addition, the study report presented mean feed consumption as g/rat/day only for the entire study period, and these data indicate that the overall mean feed consumption was comparable to the controls for all dose levels.

4. Gross Pathology - Organ weights were determined only for dams with live fetuses at term (20, 20, 19, and 16 dams in the controls, 100, 300, and 900 mg/kg/day dose groups, respectively). At 900 mg/kg/day, absolute kidney weights were increased by 19% ($p<0.05$) compared to the controls (1.87 g vs. 1.57 g), but relative (to body weight) kidney weight data were not provided. In addition, the

Table 3. Maternal feed consumption g/100 g body weight[a]

Interval	Dose in mg/kg/day (# of Dams)			
	Control (20)	100 (20)	300 (19)	900 (16)
Pretreatment: Days 0-7	9.27	9.00	9.14	9.03
Treatment: Days 7-14	8.62	8.62	8.67	6.75*
Treatment: Days 14-17	8.33	8.34	8.36	8.59*
Posttreatment: Days 17-21	7.65	7.87	8.09	8.39*

a: Data extracted from the study report page 33.

study report states that the kidney weights were increased compared to the historical controls, however, data to support this statement were not provided. Heart, liver, and spleen weights were comparable to controls at all dose levels and kidney weights were comparable to the controls at the low and mid dose levels.

Gross necropsies were performed on all dams in the study. A significant degree of general autolysis occurred in one high dose dam that was found dead on study. Of the other animals examined, gross pathology of the kidney was noted in all dose groups but not in the controls and was characterized as slight, medium, or marked dilation of one or both renal pelves. These findings were noted in 1 dam at 100 mg/kg/day, 2 dams at 300 mg/kg/day, and 1 dam at 900 mg/kg/day. Although the renal pathology was observed at a low incidence in all groups and there was a lack of a dose-response from the low- to high-dose, this finding was not considered supportive of other evidence of treatment-related renal toxicity.

5. Cesarean Section Data - Cesarean section observations are found in Table 4. The numbers of corpora lutea, implantations, viable fetuses, and the extent of pre-and post-implantation losses were similar between control and treated groups. Fetal and placental weights were also unaffected by treatment.

One female at 300 mg/kg/day and 3 females at 900 mg/kg/day were found to have empty implantation sites in the uterus at necropsy. Two of the animals in the high-dose group that had total litter loss were found to have bloody secretions in the vagina on gestational days 13 or 15. The other dams that had total litter loss (one mid-dose and one other high-dose dam) were not observed to have bloody vaginal secretions at any time. The study authors concluded that the incidence of total litter loss in the high-dose group was treatment-related. In the mid-dose group,

however, the study authors concluded that as <10% of the dams had empty implantation sites (the stated maximum incidence of this finding in the historical controls), this was not a treatment-related finding.

B. DEVELOPMENTAL TOXICITY

1. External, Visceral, and Skeletal Examinations: Fetal examinations included external, internal, and skeletal observations. Fetal findings were classified as malformations (major defects), minor anomalies, variations, or growth retardation. The study report did not provide an overall summary for the number of fetuses and litters affected in each evaluation category. However, summaries were provided for the incidence of normal fetuses and litters observed. Tables 4a, and 4b summarize the most common findings.

There were no malformations observed in any of the treatment groups. All of the minor anomalies, variations, and signs of growth retardation were observed at incident rates that were statistically comparable to the concurrent controls with the exception of the fetal incidence of uni- or bilateral wavy and/or thickened ribs (14.6% in high-dose group vs. 4.5% in controls, $p<0.05$) and unossified metacarpal 5 of the forepaw (14.6 and 14.5% in low- and mid-dose groups vs. 5.3% in controls, $p<0.05$). The study authors concluded that neither of these findings were treatment-related because the incidence of both findings were within historical control ranges. However, the increased incidence of wavy and/or thickened ribs suggests a treatment-related finding. Both the fetal and litter incidence were increased in the high-dose group relative to concurrent controls, although the difference was not significant on a litter basis (37.5% vs. 25.0% in controls). As the historical control data reported were only as a range of fetal incidence and the individual study data and litter incidences were not reported, a useful comparison with the study data was not possible.

The increased incidence of unossified metacarpal 5 of the forepaw in the low- and mid- dose groups is not considered treatment-related as it was not dose-related.

Table 4. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	100	300	900
# Animals Assigned (Mated)	20	20	20	20
# Animals Pregnant	20	20	20	20
Pregnancy Rate (%)	(100)	(100)	(100)	(100)
# Nonpregnant	0	0	0	0
Maternal Wastage				
# Died	0	0	0	1
# Died Pregnant	0	0	0	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	303	272	289	228
Corpora Lutea/Dam	15.2±2.2	13.6±1.2	15.2±2.2	14.3±1.4
Total # Implantations	267	241	256	211
Implantations/Dam	13.4±2.2	12.1±3.1	13.5±1.2	13.2±1.8
Total # Litters	20	20	19	16
Total # Live Fetuses	258	227	249	196
Live Fetuses/Dam	12.9±2.6	11.4±3.1	13.1±1.5	12.3±3.0
Total # Dead Fetuses	not reported	not reported	not reported	not reported
Dead Fetuses/Dam				
Total # Resorptions	9	14	7	15
Early	9	14	6	14
Late	0	0	1	1
Resorptions/Dam	0.45±0.83	0.70±0.66	0.37±0.76	0.94±1.57
Early	0	0	0.32±0.75	0.88±1.54
Late	0	0	0.05±0.23	0.06±0.25
Litters with Total Resorptions	0	0	1	3
Mean Fetal Weight (g)	3.2±0.2	3.4±0.4	3.3±0.2	3.3±0.3
Males	not reported	not reported	not reported	not reported
Females				
Crown to Rump Length (mm)	35.3±1.0	35.6±1.6	35.4±1.1	35.4±1.5
Sex Ratio (% Male)	45.7	44.1	49.8	51.0
Preimplantation Loss (%)	9.86	12.2	10.3	7.49
Postimplantation Loss (%)	4.46	6.06	2.79	8.76

a Data extracted from the study report pages 36 and 37.

Table 4a. Summary of noteworthy fetal observations at necropsy & skeletal examination[a]

Observations	Dose (mg/kg/day)				Historical controls [b]
	0	100	300	900	
#Fetuses (litters) examined	133 (20)	117 (20)	130 (19)	103 (16)	---
EXTERNAL/VISCERAL					
#Normal fetuses (litters)	131 (20)	113 (20)	130 (19)	102 (16)	---
Minor anomalies[c]:					
Kidney- distended right pelvis	1.5 (10.0)	0.9 (5.0)	0 (0)	0 (0)	---
Retarded growth[c]:					
Retarded fetus	0 (0)	1.7 (10.0)	0 (0)	1.0 (6.3)	---
SKELETON					
#Normal fetuses (litters)	23 (10)	20 (10)	50 (16)	24 (10)	---
Minor anomalies[c]:					
Ribs- wavy and/or thickened, uni-or bilateral	4.5 (25.0)	6.0 (20.0)	6.9 (26.3)	14.6* (37.5)	0-18.6 (---)
Variations[c]:					
Extra vertebra or rib- anlage of a 14th thoracic vertebra and an analogous 14th rib, bilateral	1.5 (10.0)	3.4 (10.0)	0 (0)	1.9 (12.5)	---
Extra rib- at 1st lumbar vertebra, uni- or bilateral	36.1 (85.0)	43.6 (90.0)	18.5 (68.4)	41.7 (87.5)	---
Retarded growth[c] :					
Skull- unossified or partially ossified bones	51.1 (95.0)	41.9 (75.0)	40.0 (89.5)	39.8 (75.0)	---
Caudal vertebra- <2 centers ossified	9.0 (25.0)	9.4 (30.0)	3.8 (21.1)	7.8 (25.0)	---
Sternebra- unossified or partially ossified	29.3 (85.0)	35.0 (75.0)	23.8 (73.7)	29.1 (75.0)	---
Forepaw- unossified metacarpal5	5.3 (30.0)	14.5* (30.0)	14.6* (42.1)	3.9 (18.8)	2.9-67.0 (---)
Hindpaw- phalanx III of 1st to 5th toe unossified	5.3 (25.0)	8.5 (20.0)	3.8 (26.3)	2.9 (12.5)	---

a Data extracted from the study report pages 38-40.

b Historical control values are for the range of % fetal incidence only and were given only for findings which were observed at statistically increased incidence in one or more of the treatment groups.

c Percent affected [Fetal (litter) incidence]

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Table 4b. Summary of noteworthy fetal observations at body cross-sectioning[a]

Observations	Dose (mg/kg/day)			
	0	100	300	900
#Fetuses(litters) examined	125(20)	110(20)	119(19)	93(16)
EXTERNAL/VISCERAL				
# Normal fetuses(litters)	124(20)	106(20)	118(19)	90(16)
Minor anomalies[b] :				
Kidney-distended right pelvis	0.8(5.0)	1.8(10.0)	0.8(5.3)	0(0)

a Data extracted from the study report page 41.
b Percent affected [Fetal (litter) incidence]

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS The study report concluded that oral administration of Hoe 061517 at 900 mg/kg/day to pregnant rats during organogenesis was associated with treatment-related clinical signs of toxicity (persistent piloerection and/or increased urinary output), reduced feed consumption, delayed body weight gain, and increased maternal mortality and incidence of total litter loss. No teratogenic effects were noted at any dose level. Oral administration of Hoe 061517 at 100 and 300 mg/kg/day produced no maternal or developmental adverse effects.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following oral administration of the test substance, Hoe 061517 (99.6% a.i.) at 900 mg/kg/day, maternal toxicity was demonstrated by treatment-related clinical findings, which included persistent piloerection (lasting 1 to 6 days) and/or increased urinary output and an increase in absolute kidney weights (19%). One of the 900 mg/kg/day dams was found dead on day 11 of gestation and general autolysis occurred. In addition, at 900 mg/kg/day, there was also a reduction in body weight gains (41%) and food consumption (22%) during the first week of treatment. However, the initial decrease in feed consumption during gestation days 7-14 was followed by a statistically significant rebound afterward (↑3 and 10%, $p < 0.05$, days 14-17 and 17-21, respectively).

In the 300 and 100 mg/kg/day groups, there were no treatment-related deaths, clinical signs of toxicity, gross findings, or changes in body weight gains, feed consumption, and organ weights observed. Low incidences of dilated renal pelvis in all treatment groups were consistent with other evidence of renal toxicity. Therefore, Maternal LOEL was 900 mg/kg/day based on treatment-related clinical signs (piloerection and/or increase urinary output), reduced body weight gains and food consumption, and increased kidney weights; NOEL was 300 mg/kg/day/day.

2. DEVELOPMENTAL TOXICITY: No treatment-related developmental effects were noted at any dose level with the exception of the increased incidence of total litter loss and the increased fetal incidence of wavy and/or thickened ribs at 900 mg/kg/day. Total litter loss seen in 3/19 dams of the 900 mg/kg/day group was a treatment-related effect. The increased fetal incidence of wavy and/or thickened ribs at 900 mg/kg/day was statistically significant (14.6% vs. 4.5% in concurrent controls, $p < 0.05$). The litter incidence of wavy and/or thickened ribs was also increased in the high-dose group relative to concurrent controls, but the difference was not statistically significant (37.5% vs. 25.0% in controls). **Based on these findings the developmental toxicity LOEL was 900 mg/kg/day; NOEL, 300 mg/kg/day.**

C. STUDY DEFICIENCIES : There are several deficiencies in this study report including (1) the historical control data was incomplete in terms of fetal morphological findings and presented in a format that was not too useful for comparative analysis and (2) gravid uterine weights and the relative kidney weights were not presented. However, these deficiencies did not affect the interpretation and analysis of the results in determining the developmental and maternal toxicity of the test animals. In addition, this study was submitted for verification of the NOEL and LOEL provided by the registrant to show that toxicity of various metabolites is less than that of the parent compound. Under the circumstance, this study is considered as **ACCEPTABLE/NON-GUIDELINE** for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in rats.

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